JULY, 1959
Vol. 13, No. 7

FT food technology

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FT food technology

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THE TECHNICAL ARTICLES IN BRIEF

Prevention of bitterness *Orange juice*

Effect of method of extraction of juice on the development of bitterness in preserved orange juice. G. S. Siddappa and B. S. Bhatia.

Gentle extraction of the juice from Coorg oranges helps in avoiding the incorporation of bitter constituents which are present mostly in the rag and peel of the fruit. Although the yield of juice can be increased by about 4–5% by heavy pressing, the resulting juice has a tendency to become slightly bitter. The extract of rag is intensely bitter, but the juice from the sacs only is free from any bitterness. Lye treatment of the segments removes bitterness, but the yield of juice is lowered slightly. The retention of ascorbic acid in bottled orange juice is as high as 65–75% during a storage period of one year at ordinary room temperature 24–30° C. To avoid bitterness in the juice, the method of extraction should be such that very little of the rag or peel gets into the juice during extraction. The addition of 5–6% of sugar to the juice helps to mask any trace of bitterness than might still get into it. (See page 349.)

Radiation preservation *Effect on color, chlorophyll of green beans, broccoli*

Effects of gamma ray irradiation on color and chlorophyll retention in green beans and broccoli. T. Wishnetsky, G. E. Livingston, F. J. Francis, and I. S. Fagerson.

Effects of gamma radiation upon color and pigment content of green beans and broccoli have been described. With a 1.86 megarad dose, a marked change in hue from green toward yellow took place in both vegetables. Irradiation produced a decrease in chlorophyll content and an increase in pheophytin. In green beans, destruction of chlorophyll amounted to about 40% of the amount originally present. With broccoli, approximately 28% of the chlorophyll content of the non-irradiated vegetables was destroyed. There was little, if any, further decrease in the chlorophyll content of either vegetable during post-irradiation storage, except for samples whose pH had been raised. A decrease in the pheophytin content of both vegetables occurred during the latter part of storage. Green beans and broccoli were slightly greener after one year's storage. Irradiation in the frozen state produced brighter, greener samples with a higher chlorophyll content. Packing green beans and broccoli in brine with a higher pH value prevented much of the chlorophyll breakdown and color loss. This improvement was retained to some extent with broccoli upon storage but not with green beans. Use of plain tin instead of enameled cans, under certain conditions, resulted in poorer color retention in both broccoli and green beans. However, chlorophyll content was higher in irradiated green beans packed in plain tin cans. Green beans packed under vacuum or nitrogen showed increased chlorophyll retention and less yellowing than the air packed samples. The reverse was true for broccoli. The combination of conditions which provided the greatest degree of protection against change in color and chlorophyll content consisted of immersion in high-pH brine, vacuum-packing in plain tin cans, and irradiation in the frozen state. A linear decrease in chlorophyll content of both green beans and broccoli resulted from increases in gamma radiation dose, over the range of 0.49 to 7.29 megarads. (See page 352.)

Radiation preservation *Effect on color, carotenoid retention of several vegetables*

Effects of gamma ray irradiation on carotenoid retention and color of carrots, sweet potatoes, green beans and broccoli. R. Franceschini, F. J. Francis, G. E. Livingston, and I. S. Fagerson.

This study is concerned with the retention of carotene and xanthophyll in green beans and broccoli; color, total carotenoids, and beta-carotene and xanthophyll in sweet potatoes; and color total carotenoids, beta-carotene, alpha-carotene and xanthophyll in carrots when irradiated under a variety of conditions. It was found that the destruction of carotene and xanthophyll in green beans upon irradiation ranged from 5 to 95% depending on the conditions. Greatest loss was found in beans packed dry in air and irradiated frozen. Least destruction was found in samples packed in brine under vacuum irradiated at room temperature and stored at 100° F. Destruction of carotene and xanthophyll in broccoli ranged from 25 to 50%. The greatest retention was found with samples packed dry under nitrogen and irradiated in the frozen state. Destruction of carotenoids in sweet potatoes ranged from 3 to 20%. Samples with the greatest retention were packed in water under vacuum and irradiated at room temperature. Destruction of carotenoids in carrots ranged from zero to 56%. The samples with the greatest retention were packed in brine under vacuum in plain tin cans and irradiated in the frozen state. These conditions also resulted in the most attractive color for both carrots and sweet potatoes. Upon storage, there was usually an initial

JULY COVER. The July cover shows the IFT Council Meeting in session in Philadelphia, May 17, 1959, at the Nineteenth Annual Meeting of the Institute of Food Technologists. Space limitations prevent listing the names of the 57 persons shown, but standing, left to right, are Arthur N. Prater, Treasurer, Aksel G. Olsen, President, 1958–59, Ray B. Wakefield, president 1959–60, and Charles S. Lawrence, Executive Secretary. Seated, continuing from the right and second from Colonel Lawrence, is I. J. Hutchings, President-Elect, 1960–61. In the left-hand corner of the photograph, seated, are four former IFT presidents Emil M. Mrak, La Verne E. Clifcorn, Philip K. Bates and George F. Garnatz. The photograph was provided through the courtesy of the Standard Photo Service Co., 2031 Chestnut St., Philadelphia.

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decrease in pigment content followed by a rise and then a slow decline. The hues of sweet potatoes and carrots gradually changed towards the yellow upon storage and samples became darker. Temperatures of irradiation, headspace gas, packing media, can lining and temperature of storage all had a small effect on the color and carotenoid content of the 4 vegetables. Blanching or puréeing resulted in a much lower pigment content in irradiated sweet potatoes. (See page 358.)

Water retention *Beef muscle*

Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. C. E. Swift and M. D. Berman.

Interest in identifying factors which affect the water retention of meat has been much in evidence in the meats field. Practical goal of this research is the development of means of controlling and, when desired, enhancing the capacity of meat to retain water or juice. Present knowledge permits this to only a limited extent. A comparative study of the composition and properties of 8 different muscles from bovine animals was conducted in an investigation of factors which influence water retention. The results indicate that considerable variation exists with respect to water retention and pH and the nitrogen, fat, moisture and electrolyte content of the muscles. The relative order of the muscles of the different animals followed a pattern with regard to water retention, pH and a number of other properties. Statistically significant correlations were obtained between water retention and directly related components, including pH, zinc and fat content, and ratios of moisture to protein, and inversely related components, including protein content (N% x 6.25), and calcium, magnesium, and potassium content. A direct, highly significant correlation was found between water retention and zinc content, in contrast to the inverse relation found between water retention and either calcium or magnesium content. This indicates that zinc differs in an important aspect from the two other ions. The possibility that zinc may participate in determining pH as a component of an enzyme system is pointed out. The ratio, moisture/protein content, was found to be directly related to water retention; hence, the results show that the relative ability of the muscles to hold added moisture was predictable on the basis of the original proportions of moisture and protein present. The analysis of factors influencing water retention is a complex problem. The present results have opened new channels for investigation; specifically, one of these concerns determination of factors influencing pH, including zinc containing enzymes, and the other, the paradoxical relation noted between protein content and water retention. (See page 365.)

Antibiotic *Method for CTC in ice*

A new fluorometric determination of chlortetracycline in ice. Tetuo Tomiyama and Yasuo Yone.

Chlortetracycline-containing ice is widely used for keeping the trawler's catches fresh in the western sea area off Japan. A practical field method for determining chlortetracycline (CTC) incorporated in ice is required to establish levels of concentration, distribution, and destruction of the antibiotic in the ice. A new fluorometric method for determining CTC in ice or in solution is presented. The method was established by data showing a linear relationship between CTC concentration and intensity of fluorescence of aluminum-CTC chelate and also little interference due to presence of decomposition products of CTC. A fairly good agreement was obtained between CTC values as assayed by the microbiologic method and the proposed fluorometric method. This method can be employed for field assays of CTC concentration in ice. (See page 370.)

Process instrumentation *Canning and other food processing plants*

Supervisory control for the food processing plant. Carl W. Ohgren.

Discussed are the results and advantages derived from central station supervisory control. Such control was applied to process instrumentation installed in a vegetable canning plant and in one other food processing plant. Quality, yield, and flexibility effects are presented. Under this system, one supervisory operator has the responsibility of making all decisions quickly. He can do so accurately, since at a glance, most pertinent information regarding the process and raw materials are at his disposal as they occur. Results indicate that the food technologist working in the processing industry could investigate, and profitably adapt control instrumentation with central station supervisory control. Petroleum refineries, chemical plants and certain corn refining plants have used this type of control during the past 10 years. Comparative cost of installing process instrumentation with and without a supervisory control in new plants as well as the comparative cost of converting existing plants to supervisory control are presented. (See page 374.)

Bacteriology *Frozen coconut*

Bacteriological flora of frozen coconut. L. A. Nutting, R. A. Larson and F. W. Barber.

Coconut-meat is sometimes marketed in the frozen state as a coconut-sucrose mixture (3 parts coconut, 1 part sucrose). Preliminary findings from primary plating of frozen coconut-sucrose samples on desoxycholate lactose agar yielded bacterial colonies characteristic of coliform types. The original findings were extended with additional taxonomic studies. Ninety-nine per cent of the positive reactions obtained by conventional coliform determinations on fresh coconut were false-positive reactions for fecal coliforms. Two reasons are evident for the occurrence of these reactions: First, the presence of fermentable carbohydrate other than lactose; second, the presence of lactose fermenting organisms which are not true indicators of fecal contamination. About 15% of the false reactions appeared to be due to organisms pathogenic for plants. (See page 378.)

Preference *Food combinations*

Measurement of preferences for food combinations. Jan Eindhoven and David R. Peryam.

The hypothesis that preference for a food combination (meat and potatoes, potato and other vegetable, etc.)

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differs from the sum or weighted average of the individual component preferences was investigated in a pilot study. Questionnaires requiring ratings on how well the foods of a pair go together were completed by 593 military personnel. One important variable studied was form length; forms containing 50 or 100 combinations were used. Three food classes were used for the combinations: meats, potatoes, and vegetables. Results of the study indicated that how well main dishes and potatoes go together, and how well main dishes and vegetables go together, are in large part independent of preferences for the individual components. Potato-vegetable combination effects were not generally found, but this may have resulted from the specific foods paired. Differences between forms generally had little or no effect on the food combination effects. The investigation of combination preferences by a questionnaire method was shown to be feasible. (See page 379.)

Objective analysis *Measurement of volatile reducing substances in coffee*

The influence of high temperature holding upon the components of coffee brew. II. Volatile reducing substances. Stanley Segall and Bernard E. Proctor.

A reliable means of objectively evaluating coffee aroma and flavor has long been sought. In this study the volatile reducing substances content of standard coffee brew was determined by the method of Lang, Farber, Beck, and Yerman. It was found that volatile reducing substances were sensitive to the length of time coffee brew was held. The data showed that this class of compounds decreased rapidly on holding, even at the lower holding temperatures. It is concluded that the volatile reducing substances content is an indication of the age and treatment afforded a coffee brew. It should be possible to utilize this factor in establishing standards for coffee brew, and as an objective means for supplementing organoleptic evaluation. (See page 383.)

Grade, size of apple *Processing of apple slices*

Relationships of apple grade and size to apple value in processing of apple slices. Joseph M. Johnson, Anthony Lopez, Charles B. Wood, and Roy E. Moser.

A correlative study with that previously done for applesauce processing was completed for processing apple slices in the 1957–58 processing season. The study was designed to determine the effects on recovery of apple fractions and preparation time requirements of grade and size of fruit in processing of apple slices under commercial conditions. The present United States grades No. 1 and No. 2 and three experimental grades proposed by the Virginia Horticultural Society's Industry Advisory Committe in each of three size classes were studied. Estimating equations were derived from the experimental data by a statistical procedure known as differential contrast to estimate the recovery of peels and cores, total trim, defect trim, broken slices, and prepared apple slices when apples of the different grades and sizes were utilized. The trimming time requirements for the different grades and sizes were also estimated. Average yields of apple fractions and trimming time requirements for the 4 plants included in the study are shown. These values may be used to determine

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the relative value of the different grades and sizes of apples by utilizing the methods shown in the previous study of applesauce processing. The refining of apple grades indicated as being to the advantage of high quality large apple size producers points also to a need for a refinement of our apple size classifications. The variation in size that occurs within a ¼ inch range in size is sufficient to materially affect both recovery of prepared product and preparation time requirements. A measurement of size on the basis of the number of apples per 100 pounds of fresh fruit is suggested as an alternative to the present diameter size classification. (See page 385.)

Enzymatic discoloration *Onions*

Pinking of onions during dehydration. Thomas M. Lukes.

During the commercial production of dehydrated onions one of the problems is the development of a pink pigment in the onion during the dehydration process. This investigation as to the causes has demonstrated it is not due to bacterial action. The actual course of the reaction is as follows: When the onion cells are reuptured, the native enzymes react with a substance in the juice to form a color developer. This then reacts non-enzymatically with the amino-acids in the onion to form a pink pigment. (See page 391.)

Chemical analysis *Carotenoid pigment changes*

Changes in the carotenoid pigments in preparation and storage of Valencia orange juice powder. A. Laurence Curl and Glen F. Bailey.

A study was made of the changes in the carotenoid fraction during the preparation and storage of powdered Florida Valencia orange juice. The composition of the carotenoid mixtures from frozen single-strength and concentrated juices showed no significant differences indicating that the concentration process had little or no effect. In the preparation of powdered orange juice a minor part of the carotenoid 5,6-epoxides, which compose about ⅔ of the pigments, was isomerized to 5,8-epoxides. This change occurred to a much greater extent on conditioning the powder at 77° F. for 78 days. On subsequent storage at 100° F. for 6 months, the 5,6-epoxides practically all disappeared and appreciable decreases were found in some of the non-epoxide carotenoids, which may be, at least in part, accounted for by trans-cis isomerization. Most of the apparent decrease in total carotenoid content, when measured colorimetrically at 440 mμ or spectrophotometrically for individual constituents can be accounted for by these isomerizations. No losses were observed in the provitamins A (cryptoxanthin and β- and α-carotenes) during the conditioning and aging periods. The composition of the carotenoid mixture from Florida and California Valencia orange juices was similar, though the total content was lower in the Florida juice. Two very minor components obtained from Florida juice which had not previously been found in California juice, were apparently lutein 5,6-epoxide and the corresponding 5,8-epoxide flavoxanthin. (See page 394.)

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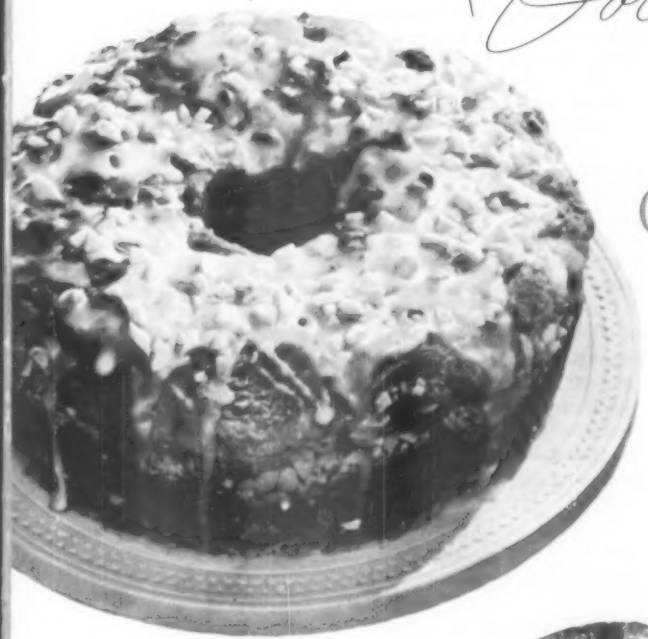

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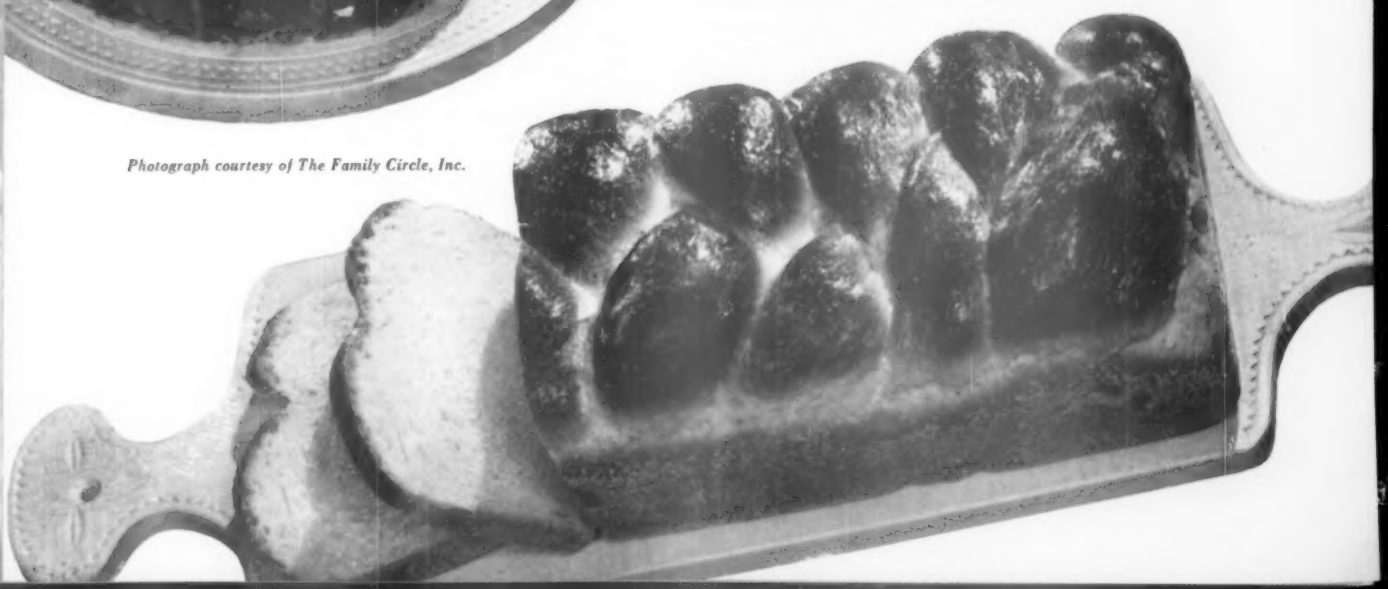

Radiation preservation — *Chicken meat, sweet potatoes*

Storage characteristics of some irradiated foods held at various temperatures above freezing. I. Studies with chicken meat and sweet potatoes. J. J. Licciardello, J. T. R. Nickerson, B. E. Proctor and C L. Campbell.

Storage studies were run on irradiated chicken and sweet potatoes held at temperatures ranging from 50° F. to 125° F. for 8 to 10 months. Products stored at 50° F. and at 68° F. were still regarded as acceptable after 8 to 10 months. Deterioration in quality occurred within one month when samples were stored at 125° F. With irradiated chicken, shelf life was limited by: proteolytic enzyme action, which resulted in poor texture; non-enzymatic browning, which caused undesirable color changes and presumably imparted a bitter off-flavor; development of oxidative rancidity, particularly at the lower storage temperatures. Internal corrosion of the metal container occurred during storage of irradiated sweet potatoes. This produced a metallic flavor in the product and was the chief cause of loss of quality. Vitamin analysis for thiamin, riboflavin and pyridoxine indicated that the amount of these vitamins destroyed by a sterilizing dose of cathode rays was comparable to the amount destroyed by conventional methods of thermal processing. Loss of vitamins in irradiated samples or heat-processed samples during storage at similar temperatures proceeded at about the same rate during storage. (See page 398.)

Radiation preservation — *Pork sausage and scallops*

Storage characteristics of some irradiated foods held at various temperatures above freezing. II. Studies with pork sausage and scallops. J. J. Licciardello, J. T. R. Nickerson, B. E. Proctor, and C. L. Campbell.

Storage studies were carried out with irradiated pork sausage and scallops held for 8 to 10 months at temperatures ranging from 50° F. to 125° F. Even after 8 months' storage at 50° F., irradiated pork sausage was considered acceptable in flavor. At storage temperatures of 68° F. or above, irradiated samples became unacceptable within 2 months. Irradiated scallops were considered to be acceptable after 10 months' storage at 50° F. or 68° F.; however, a marked deterioration in quality occurred within one month in samples held at 125° F. Non-enzymatic browning, which caused off-flavor and color degradation, played the principal role in deterioration of irradiated scallops during storage, but texture losses also occurred. In regard to irradiated pork sausage, texture change and associated off-flavors due to proteolytic enzyme action chiefly contributed to loss of quality. Color changes and fat oxidation also occurred. (See page 405.)

Heat inactivation of enzyme — *Peroxidase*

Heat inactivation of peroxidase in HTST processed whole kernel corn. J. L. Vetter, A. I. Nelson, and M. P. Steinberg.

Heat inactivation of sweet corn peroxidase was studied under semi-commercial conditions by heating brine packed whole kernel corn in Number 1 cans in a pilot model end-over-end agitating cooker Heat inactivated samples showed a tendency to regenerate activity when stored for 72 hours at 75° F. Heat inactivation time curves for peroxidase were characterized by a change in slope at about 270° F. The heat inactivation time curve based on samples analyzed immediately after heating showed at F_{∞} value of 3.50 minutes and a F_{∞} value of 0.57 minutes. The z value of the line for temperatures below 270° F. was 49° F.; the z value for temperatures above 270° F. was 70° F. When samples were allowed to regenerate during 72 hours storage at 75° F., the heat inactivation time curve exhibited a F_{∞} value of 7.00 minutes and F_{∞} value of 2.94 minutes. The z values were 70° and over 300° F. for temperatures below and above 270° F., respectively. It was found that regeneration of activity in heat inactivated peroxidase samples began immediately after heating and that it was more rapid and extensive in samples containing a small amount of residual enzyme activity immediately after heating than in samples showing no residual activity at that time. The theoretical significance of these results is discussed (See page 410.)

Spectrophotometric method — *Tomato ketchup adulterants*

A rapid spectrophotometric method for the detection and estimation of adulterants in tomato ketchup. Om Parkash Beerh and G. S. Siddappa.

A rapid method has been evolved for the estimation of lycopene in tomato products based on the measurement of absorption at 503 mμ using the petroleum ether extract of the total carotenoids. The replacement of tomato solids in tomato ketchup by such adulterants as carrot, ash gourd, pumpkin, sweet potato, papaya and apple pulps lowers the lycopene content of the product. On this basis the tomato solids content of the product has been determined by estimating its lycopene content. Adulterants like carrot pulp increase considerably the β-carotene content of the ketchup. There is very little loss in the lycopene content of tomato ketchups, genuine as well as adulterated, during a storage period of 2 months at 37° C. A lycopene index of 475 μg./g. of tomato solids has been tentatively proposed for the estimation of the extent of adulteration in tomato ketchup, when other fruit and vegetable pulps are employed as adulterants. (See page 414.)

Acidification — *Canned tomatoes*

The pH problem in canned tomatoes. S. Leonard, Rose Marie Pangborn, and B. S. Luh.

Acidification of canned tomatoes with citric acid is suggested as a means of increasing acidity to a safer and more desirable level. Because additions of acid (0.1, 0.2 and 0.3%) increased the sourness of the product, additions of sucrose were needed for compensation. The data point out the variability of total acids, and pH of individual samples. A considerable amount of additional research on the pH problem is needed. Specifically, further information is needed on microbiological changes, sterilization requirements, processing procedures, drained weight considerations, storage stability and finally, consumer acceptance. (See page 418.)

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416-P

Flavoring Agents as Food Additives*

Richard L. Hall
McCormick & Company, Inc.,
Baltimore, Maryland

FLAVORS FORM by far the largest and most complex problem area in the field of food additives. Approximately 1,100 flavor additives and adjuncts are now in use in food. This is probably about twice the number of additives in use for all other purposes. It would not be minimizing the difficulties facing other food additive areas to say that no single group of additives poses problems that are at once as numerous, as difficult, and as unusual as those of the flavor field. Here, as elsewhere, the Food and Drug Administration has made a good beginning on their unenviable task. We are confident that the continuing cooperation of industry and the Administration, and the help of independent groups such as the Food Protection Committee, will provide reasonable solutions to these problems. We will, however, need a resourceful and flexible approach which, without evading statutory requirements, meets the particular needs of this situation. It would be impossible in a discussion of this type to do more than to illustrate briefly certain of these unusual aspects of the flavor additive situation; and, in the time that follows, I would like to touch on three of these topics.

LEVELS OF USE

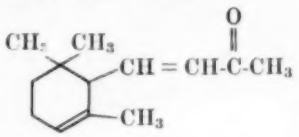
The first of these is the problem of usage at levels which are functionally effective, but are insignificant from a toxicological and public health point of view. An interesting review of this subject entitled "A Report on Insignificant Levels of Chemicals in Food" was made by an industry advisory subcommittee to the Food Protection Committee in November, 1957. The principles set forth in this paper can be extended to the question of flavor additives, but the subject of flavor additives raises complications which go beyond the conclusions of the subcommittee's report. The report assumes the existence of fairly detailed knowledge of the toxicology of the substance in question, and goes on to conclude that usage at levels far below the "safe" level may reasonably be ignored. Such detailed toxicological information simply does not exist on hundreds of flavor additives. Yet many of these same additives play an extremely important part in the final food product at levels of use which cannot be of toxicological signficance almost without regard for the other factors involved. I should like to support this statement with a few specific examples.

First is the substance, methyl heptine carbonate.

$$CH_3CH_2CH_2CH_2CH_2C \equiv CCOOCH_3$$

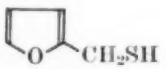
It is used principally as an important secondary constituent of imitation strawberry flavor to which it contributes a "green" note. Its concentration in the finished foods in which it is used ranges from 0.0018 part per million to 1 part per million, with a usual figure of around 0.1 part per million. The total national annual intake of this material in foods and beverages cannot exceed 50 pounds per year. Assuming a national food consumption of 262,000,000,000 pounds per year *(4)*, this is a concentration in the total diet of 0.2 part per billion. Foods flavored with imitation strawberry flavor are not uncommon; but even in extreme cases, they could not form more than half the total diet, and the concentration in the diet of even an abnormal individual could not be more than a small fraction of a part per million.

Another example is α-ionone.



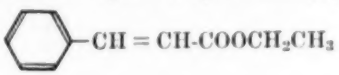
This is used in imitation raspberry flavor at a level that ranges from about 0.1 to 2 parts per million, with an average of somewhat less than 1 part per million. No more than 250 pounds of this material is used in all foods and beverages in a year. This amounts to a proportion of 1 part per billion in the total diet.

Furfuryl mercaptan



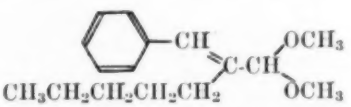
is a critically important ingredient of imitation coffee flavor. This is used at a level which ranges from 0.01 to 1 part per million. The total national usage is perhaps as high as 50 pounds per year. Again, the concentration in the total diet is approximately 0.2 part per billion.

Still another example is ethyl cinnamate,



used in imitation vanilla and strawberry flavors at levels which range from 0.02 to 0.75 part per million. An estimate of total national usage for this compound would be 500 pounds, equivalent to 2 parts per billion in the overall diet.

A final example is amyl cinnamic aldehyde dimethyl acetal.



In minute quantities, it imparts a "deep green" note to certain imitation fruit flavors. Usage levels in finished food products range from 0.1 to 0.5 part per million. Total annual national consumption is about 25 pounds per year.

* Presented at the Symposium on Food Additives, Annual Meeting, Institute of Food Technologists, Bellevue-Stratford Hotel, Philadelphia, May 20, 1959.

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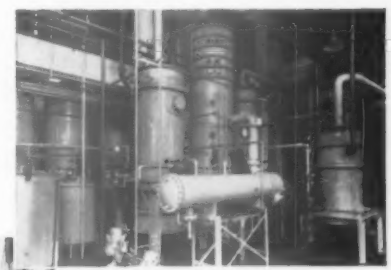

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There are many other examples of this sort; and these represent a large and important group of flavor additives. It may, at first, be argued that levels of use so low must necessarily imply that the ingredient cannot be of critical importance. This raises a number of serious questions which cannot be answered easily. Character and quality in flavor do not admit of easy description and definition. Taste panel measurements are at best an approximate science—a tool which is a useful guide. But who is to determine the commercial importance of a superior flavor as compared to a merely adequate one? Flavor compounding is a mixture of intuition, science, experience, and—let us be candid—hocus-pocus. But the factors of skill and experience are great. The question of whether or not a particular flavor ingredient is important is not a question on which the opinion of an untrained individual is entitled to serious consideration. Let me hasten to add that I am not an expert in this area, and must, therefore, rely for guidance on those who are.

Most important of all, however, we should maintain a sense of perspective in viewing this problem. The American public inhales each year perhaps 30,000 pounds of solid air-borne pollutants and many times this quantity of gaseous pollutants *(1, 3, 8, 9)*. We consume 400,000 pounds of pyridine, in addition to other interesting substances in coffee *(6, 10)*. We drink about 1,500,000 pounds of fusel oil in distilled liquors *(2, 7, 11)*. We also inhale about 14,000,000 pounds of tars from tobacco *(5, 7)*. I do not quote these figures in order to view them with alarm, or to shift concern elsewhere, but merely to call attention to the level of consumption of certain substances which we readily accept, even though their safety "under conditions of intended use" is at least open to some question. It would be difficult to maintain that the full array of tests of toxicological safety should be brought to bear on those flavoring substances, whose annual usage is of the order of a thousandth to a millionth of these other substances whose consumption remains without effective control. The principle of "de minimus non curat lex" (the law takes no account of trifles) has a sound basis both in law and common sense. There are many food additives, including not a few flavoring substances, which are used at levels and in such volume as to demand the most careful scrutiny. Among food additives, these must be the first and remain the principal concern of the technical and regulatory facilities of both industry and government.

THE REQUIREMENT REGARDING ANALYTICAL METHODS

The second area of special difficulty with flavors is the requirement of the Food Additives Amendment, spelled out in somewhat more detail in the regulations, for a practicable analytical method. This is generally interpreted to mean an analytical method capable of determining the presence of a given additive, at least qualitatively, and preferably semi-quantitatively, at the maximum level of use permitted in food. Applied to insecticidal residues, preservatives, emulsifiers, etc., this is a reasonable requirement, justified not only, in some cases, by the potential dangers of overuse, but by their volume of use and the technical feasibility of detecting such substances in food. The actual application of this provision to flavors has not been worked out in practice, but if it were applied literally to all flavor ingredients, it would often be unrealistic and uneconomic and, in some cases, impossible. Modern analytical tools are capable of performing feats of incredible sensitivity and selectivity, but the development of detailed methods for using them for the isolation, identification, and quantitative measurement of a given compound poses problems which are exceedingly tedious and expensive to solve. The development of analytical methods may well exceed the cost of full-scale animal feeding tests. It is obvious that flavors whose annual usage may be a few pounds or even a few hundred pounds a year cannot support the economic consequences of these technical requirements. Finding ethyl cinnamate or α-ionone, even qualitatively, in the final food in the presence of many other ingredients of similar structure and chemical characteristics is a technical problem of first magnitude. It would not seem reasonable or practicable to apply literally the requirements of the regulations to a food additive which enjoys such a low volume of use and at the levels mentioned. The value of the information supplied would not be commensurate with even a fraction of the cost of acquiring it.

We sincerely hope that in establishing and applying procedures and criteria, we can cut the cloth to fit the man, and not merely to conform to a standard pattern. For if it were to occur, the indiscriminate and literal extension of the food additive regulations to substances such as we have discussed would cripple seriously the flavor industry. It would deprive the food industry of critical components and would restrict severely the freedom and quality of choice now open to the consumer. These results would follow without any significant gain to public health or individual safety. To avoid this, both the Food and Drug Administration and industry must have full knowledge of the facts and complete mutual understanding.

A further factor of considerable importance is the self-limiting character of many of these additives. Although generalizations, valid in each case, are not possible, most flavor ingredients cannot be used at levels of 10 or 100 times their normal maximum use without making the finished food unpalatable. This is a factor which does not operate in the case of many other types of additives, and it provides an additional safeguard against accidental or unintelligent use.

FIVE SUGGESTIONS FOR DEALING WITH FLAVOR ADDITIVE PROBLEMS

So far, these remarks have attempted to emphasize the inadvisability of applying rigidly regulations directed primarily toward additives used at moderate to high levels or in large total volume, or which present particular elements of hazard in use. In view of the complex and difficult job facing us all, some

constructively intended comment, rather than only warnings or objections, would be appropriate at this early stage of development of precedents and procedures. Although the following suggestions for dealing with some of the problems raised by flavor additives are my own, I feel certain they would have support in industry:

1) A frank recognition of the application of the *de minimus* principle where appropriate.
2) Where circumstances require more rigorous assurances of safety, the *de minimus* principle cannot be applied. In these cases, the toxicological data and analytical methods to be supplied should be no more than appropriate to the degree of potential hazard in a given case. Criteria of broad applicability are convenient to administer, but they must necessarily be directed toward coping successfully with the most difficult or hazardous cases. As we have seen, this would have a stultifying effect on an important segment of the industry without a corresponding benefit to public health.
3) Every encouragement should be given to the development and acceptance of new, shorter, less tedious and expensive procedures for demonstrating toxicological safety, and developing methods of analysis. The flavor industry is in the peculiar position of using many critical ingredients whose value is very large in terms of the characteristics they contribute to the final foods. The dollar volume sold of each additive, however, is so low that, in most cases, they could not conceivably support the animal testing and development of analytical methods envisaged by the regulations.
4) Presumptive evidence of safety may be adduced from many sources. In the absence of unfavorable evidence, and where the potential hazard is apparently small, such presumptive evidence should be sufficient. This will require general recognition that the so-called exempt lists and the regulations governing use of additives are not imperishable, but subject to change as new evidence is brought to light.
5) The flavor industry, traditionally secretive, must recognize and assume its responsibilities. The Food and Drug Administration can discharge its functions and meet the needs both of the public and industry, only if it is supplied the information that can come solely from industrial sources.

YOUR ASSISTANCE IS NEEDED

The flavor industry is currently beginning a detailed survey of the use of flavoring substances. We have obtained, through the cooperative efforts of dozens of manufacturers, a list of approximately 1,100 flavor additives and adjuncts. These substances have been reviewed and a standard nomenclatural system devised. A large number of flavoring substances,

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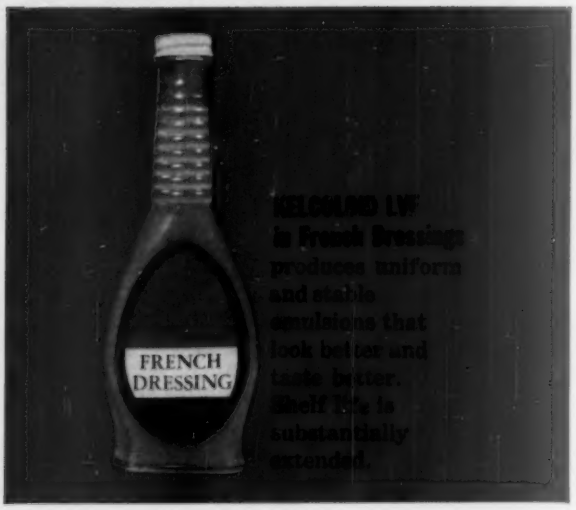

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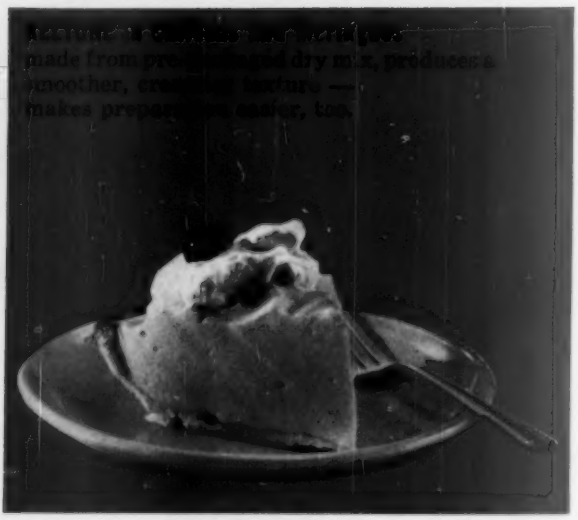

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after preliminary review, have been recommended to the Food and Drug Administration for exemption from the provisions of the Food Additives Amendment. Many others, however, are sufficiently new or unfamiliar so that exemption at this time could not be urged. In a large number of cases, the Administration has agreed with our recommendations, and has published proposals to exempt certain substances. In still other cases, they have asked for more information than was readily available.

Flavor manufacturers and users to be surveyed. In our survey, which will reach all flavor manufacturers and flavor users in many fields, we will ask that each manufacturer list for each flavor ingredient the specifications he employs, the flavors in which it is used, the earliest proveable date of use, the degree of importance which he attaches to its use, and the usual and maximum concentration in parts per million of the additive in each of several classes of finished foods. We ask, also, for the volume of use by each manufacturer, and the submission of any available toxicological data. We hope, by this means, to provide sound evidence which represents practically all available knowledge on these points. The information so compiled will be used to recommend confirmation or modification of the exempt lists, to support applications for the issuance of regulations governing the use of non-exempt additives, and, in still other cases, to provide a basis on which to judge what further information on toxicology or use may need to be developed. It will also provide a reasonable order of priority, based on commercial importance and potential hazard, if any, for dealing with these substances on which additional information is needed.

The completion of this questionnaire will be an extremely tedious job, in which we urgently request the full cooperation of all manufacturers who are asked to participate. Its tabulation—I hardly need add—will be a rather awesome task in itself. We solicit your comments and suggestions; and since many of this audience will be involved, we most urgently hope for your assistance and cooperation. Only by such a broad, cooperative program, involving the best efforts and counsel of industry, government, and the public, can our problems be surmounted successfully.

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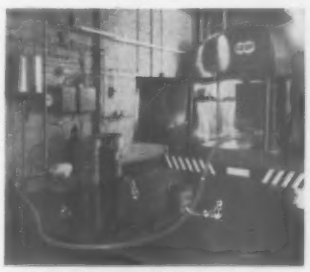

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A Technical Reference Card System*

Gilbert A. Pitman

Western-Waxide Division, Crown Zellerbach Corporation, San Leandro, California

SEVERAL REFERENCES *(1, 5, 6)* in the recent literature attest the need for literature retrieval operations in our present rapidly expanding field of technical references. Assistance from the Special Libraries Association *(2)* and even mechanical literature searching units may be indicated for the few large research organizations, but individuals and the many smaller groups have need for a less pretentious system of reviewing the recent literature. Complicated punch-card systems are available *(3, 4)*. The system herewith described has proved useful in a research group of about a dozen members whose interests extend over several "fields"—food technology, applied packaging, paper technology, wax formulations, statistical methods, etc.

HOW IT WORKS

Briefly, duplicated references are cut and pasted to 3 in. x 5 in. cards having numbered holes prepunched around the four edges. A "Master Card" like Figure 1 for each prime or first order classification shows

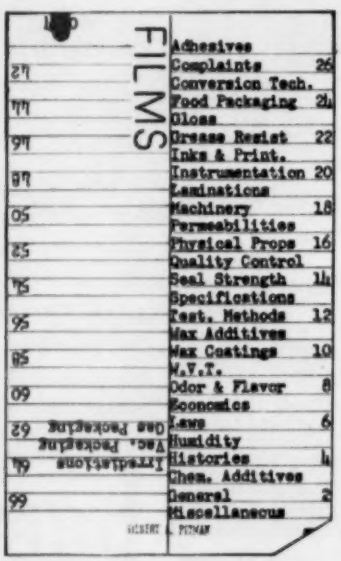

Figure 1. Typical master card.

which of these holes should be hand-notched for future rapid sorting to the prime classification and to the secondary or subclassifications. Completed cards are "filed" without regard to sequence or position. Subsequently, in searching through completed cards for references on a given subject, a hat-pin-like needle is inserted in the numbered hole assigned to the subject sought and the deck of cards shaken or riffled to dislodge those in which this hole had been opened or notched. All the cards that fall pertain to the prime classification sought, and, if these are resorted for the sub-classifications desired, a high degree of selectivity results in a greatly reduced number of references to be searched. Further, all the references encountered on this subject will be immediately at hand.

REQUISITES

Requisites for such a system to assure maximum usefulness include:

(a) An orderly method of recording references

(b) A means of providing a rapid and complete search of all received literature on a given subject

(c) Extreme simplicity of operation

(d) Cross referencing without multiple listing

(e) A system that is inexpensive to operate, both as to time and equipment.

No. 4135 E-Z sort cards (from E-Z Sort Systems, 45 Second Street, San Francisco, California) were found well adapted to this system. They have 13 prepunched holes on each 3 in. edge and 27 on each 5 in. edge. By assigning the 26 end holes to prime classifications and the 54 top and bottom holes to sub-classifications we obtained over 1400 possible classifications, which have proved more than adequate. Each of the 80 holes is sequentially numbered and so can be easily specified by the abstractor in indicating the classification and scope of an article.

Master cards are prepared by first choosing the number of *prime* classifications to be included, with provision for expansion, and assigning one of the numbered holes in the 3 in. edge to each such classification. Then a list of sub-classifications is made for each *prime* classification. Careful planning is important in assigning sub-classification numbers, e.g.: similar sub-classifications appearing on several master cards should be assigned the same numbers; alphabetical arrangements may be of advantage; and similar groupings and arrangements will prove a convenience in use. The Master Card lists are then typed with Elite type on duplicating master sheets on which lines have been drawn corresponding to the holes of a card as in the example shown in Figure 1. When duplicated, these are glued to stiff stencil board, trimmed to size, and distributed to all participating personnel.

* Presented at the Northern California Section, Institute of Food Technologists, Annual Meeting, December 6, 1957.

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ABSTRACTING

Each technical journal received is assigned to one or more staff members for review and abstraction of articles that have potential interest. Duplicate abstracts are prevented by the abstractor's initialing the title page of each article abstracted. An acceptable format for abstractions is illustrated in Figure 2, and can be summarized as follows: the title is given in caps on the first line or lines, followed by the authors and reference notations on the second and third lines. An attempt is made to restrict the texts to no more than fifty words, though longer texts can be accomplished by accordion folding or pasting on the reverse side of the file card. The text is followed by the numbers of the holes that are to be notched, the date, and the abstractor's initials.

Handwritten abstracts are collected by the stenographic group until they have enough to fill a typed page. A stencil or duplicating sheet is divided into two 4½ in. columns and the abstracts typed in these two columns. A space is left between abstracts to facilitate cutting them apart for pasting on separate file cards. Each sheet of abstracts also carries a distribution list. As new sub-classifications become necessary, the names and numbers thereof also appear on the sheet of abstracts so that all master cards will be complete.

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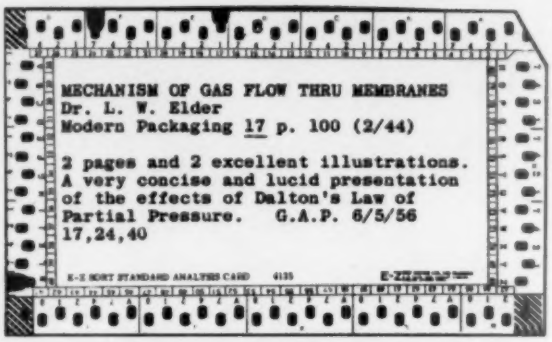
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Figure 2. Typical reference card.

One copy of every abstract is kept in the "company" file, and these cards are specially edge-colored to prevent their becoming mixed with personal files. After being pasted to the prepunched card the numbered holes listed at the bottom of the abstract are notched or opened with a special hand punch. The sheets going to the personnel on the distribution list are usually cut to remove only those items of special interest to the individual, and become their personal property to stimulate completeness of such specialty files.

It is obvious that each completed card will have at least one prime and one sub-classification hole notched. In our format this means at least one end notch and one top (or bottom) notch. A plurality of notches in either of these groups is equivalent to cross indexing. It is also obvious that the cards need not be filed in any stated sequence in the file drawer. Both of these features are important labor savers.

SUMMARY

A system is described for use by the individual or small research team in abstracting and retrieving technical references and data. Abstracts are pasted to prepunched cards which are hand notched to indicate the type of subject matter and to facilitate future retrieval operations; 1400 classifications are possible from one 3 x 5 size card. Master cards for each prime classification show the positions of sub-classifications notches. Cards sorted with long needles permit notched cards being sought to drop out of the deck. Re-sorting results in a high degree of selectivity.

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IFT NEWS

"Symposium on Foods; Enzymes" Scheduled at Oregon State in September

Related symposiums on food enzymes and molecular biology have been scheduled for September at Oregon State College with food technologists and biochemists from throughout the West expected to attend.

The "Symposium on Foods: Enzymes" will be held September 9 and 10 with the second symposium on "Perspectives in Molecular Biology" planned September 11 and 12.

The Food Technology Department is sponsoring the symposium on enzymes as the first in what is expected to become a continuing series of conferences on important food industry topics. Dr. Harold W. Schultz, head of the Food and Dairy Technology Department at the college, is symposium chairman. Purpose of the symposium is to emphasize the important role of enzymes in food processing, he noted, and new research developments on enzyme properties and actions.

Opening highlight of the symposium will be a special program honoring Prof. Ernest H. Wiegand, who founded the nation's first food technology department at Oregon State in 1919. He retired from the college staff in 1953 but is still active as a food industry consultant.

Symposium on Molecular Biology also to be held in September

The symposium on molecular biology will be the annual meeting of the Pacific Slope Biochemical Conference. Some 300 scientists from eight western states and British Columbia are expected to take part in the discussions.

This symposium will highlight the important major trends in fundamental chemical biology. It is planned in part to commemorate the completion of new research laboratories at Oregon State College, which will be devoted to basic research in the life sciences.

Dr. Vernon Cheldelin, director of the OSC Science Research Institute, will be symposium chairman.

Housing in campus dormitories is being arranged for scientists attending the two conferences. Both conferences were scheduled as a part of the Oregon Centennial Year series of scientific meetings sponsored by Oregon State College.

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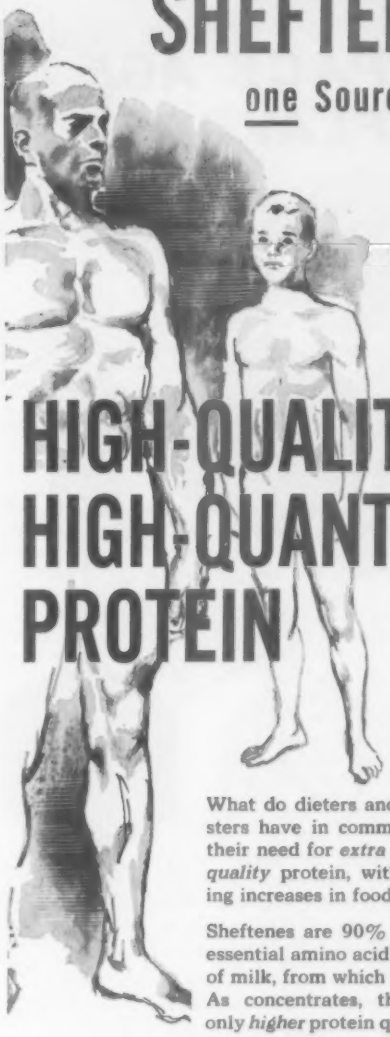

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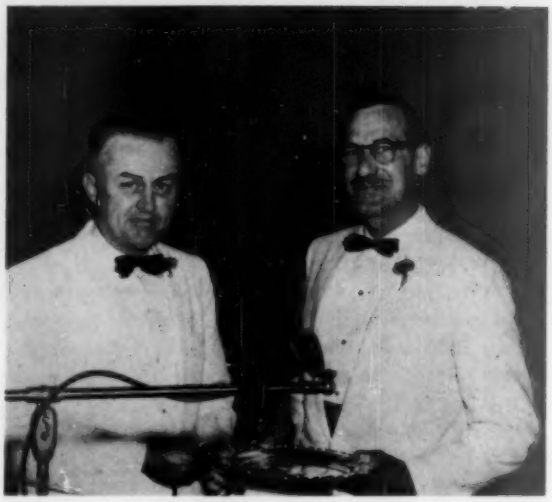

Dr. R. A. Chapman, Assistant Director, Scientific Services, Food and Drug Directorate, Canada, (right) receives the International Award for 1959, the Australian Regional Sections, Donor, at the Awards Banquet, 19th Annual Meeting of IFT, Philadelphia, May 19, 1959. Dr. Robert M. Schaffner, Vice President, Libby McNeill and Libby, (left) presented the award, an engraved silver salver.

FUTURE MEETINGS FOR FOOD TECHNOLOGISTS

June 15-Sept. 4	Gordon Research Conferences, Colby Junior College, New London, New Hampshire
August 19-26	10th International Refrigeration Congress, Copenhagen, Denmark
August 25-28	42nd Annual Meeting of the American Dietetic Association, The Statler Hilton Hotel, and Shrine Convention Hall, Los Angeles, California
1960	
May 15-19	**Twentieth Annual Meeting of the Institute of Food Technologists, Fairmont Hotel and Masonic Temple, San Francisco, California**
1961	
May 7-11	**Twenty-first Annual Meeting of the Institute of Food Technologists, Hotel Statler, New York, New York**
1962	
June 17-20	**Twenty-second Annual Meeting of the Institute of Food Technologists, Amercana Hotel, Bal Harbor, Miami Beach, Florida**

An open invitation is extended to readers of FOOD TECHNOLOGY to send in to the Editorial Office, 11606 South Bell Avenue, Chicago 43, Illinois notices of annual or national meetings of interest to food technologists.

(Continued on page 25 following technical section)

Effect of Method of Extraction of Juice on the Development of Bitterness in Preserved Orange Juice

(Manuscript received October 31, 1958)

G. S. Siddappa and B. S. Bhatia
Central Food Technological Research Institute, Mysore, India

DEVELOPMENT OF BITTERNESS in orange juice which has been heated and preserved in bottles or cans by pasteurization or sterilization is a serious drawback in processing certain varieties of oranges. With the exception of Navel oranges and immature fruit, bitterness is not known to develop in commercial varieties of oranges grown in the U. S. Marsh *(7)* has observed that Navel orange bitterness is reduced on Trifoliate *(Poncirus trifoliata)* orange root stock. Recently, it has also been observed by Australian workers that by using Trifoliate stock bitterness in orange juice can be reduced *(1)*. The chemistry of the bitter principle has been the subject of numerous investigations *(2, 3, 4, 5, 6)*. A good review of the work on the subject is presented in a Handbook prepared by the U. S. Department of Agriculture *(10)*. Several patents have been issued on the debittering of Navel orange juice, one of the more recent being that of Rockland, Beavens and Underwood *(8)*. The bitterness in the Navel orange juice is mainly due to the extraction of rag, core, segment membranes, etc., and this can be minimized by careful extraction.

In the case of the loose-jacket oranges grown in India, (*citrus reticulata*, but better known by regional names), the authors have observed that the juice of Assam oranges is remarkably free from bitterness, whereas that of the Nagpur and Coorg mandarin oranges has a marked tendency to turn bitter on heat-processing in bottles or cans *(9)*. To find out to what extent the different parts of the orange itself are responsible for this development of bitterness and to see if by suitable modification of the method of extraction of juice bitterness could be minimized—if not eliminated—a series of experiments was conducted. The main object was to evolve a simple device for the extraction of juice so that it would not turn bitter on bottling or canning. It has, however, been observed by us that lye treatment of the segments, prior to extraction of the juice, eliminates any tendency of the juice to turn bitter in the bottle or can. Further, the addition of about 5–6% sugar to the juice extracted by the usual method using a screw-type juice extractor or a hydraulic press also is of value in masking to some extent the bitterness in the heat-processed juice. The juice of *Sathgudi* orange *(Citrus sinensis)*, which is a well-known tight skinned orange, is remarkably free from bitterness.

EXPERIMENTS

Loose-jacket Coorg oranges were used for extraction of juice by different methods. For the separation of the juice of the inner portion of the orange from that of the outer portion adjacent to the peel, a simple device was adopted. It consisted essentially of a perforated cone fixed to the base of a shallow tray made of wood. A hollow cup, fitting the cone, was cut in another piece of wood. The cut half of the orange was placed in an inverted position with the juice sacs against the cone and the hollow cup placed on top of the orange. The orange was pressed between the cone and the cup. The juice from the central portion of the orange, which flowed down the perforations in the cone, was collected in a receptacle placed below; the juice from the periphery adjoining the peel, which collected at the base of the cone, was channelled into a separate receptacle. The pressed juice was thus separated into two fractions, namely, the inner juice and the outer juice. The object of this separation was to see if the bitterness of the juice is in any way due to its contact with the peel and admixture with peel emulsion. The inner juice would not be mixed with any peel emulsion, since it is drawn immediately into the pores of the cone the moment it is pressed from the juice sacs. The outer juice would naturally become mixed with some peel emulsion and also be in contact with the fibrous veins surrounding the segments. The inner and outer juices were in the ratio of 3:1 approximately. This, however, varies with the size of the cone, size of the fruit, etc. The other methods of extraction of juice were as follows:

(i) After peeling the orange, the segments were separated, placed in a piece of folded cloth and squeezed by hand, and the juice collected in a flask.

(ii) The segments were passed through a screw-type juice extractor and the juice collected.

(iii) The juice sacs were carefully separated by hand from the adhering segment cover and the juice pressed from them by squeezing in a piece of cloth.

(iv) The segments were crushed first and then pressed in cloth using a small wooden basket press.

(v) The segments were dipped for 20–30 seconds in boiling 1% caustic soda (lye) solution, rinsed immediately with sprays of cold water and then pressed in cloth to get the juice. As a result of lye treatment, the covering of the segment and the fibrous veins were completely removed, leaving undamaged the juice sacs only. There was, however, some loss of juice through dripping and washing.

(vi) After extraction of the inner and outer juices from the cut halves of the orange, the rag inside the cups was scooped out and the residual juice in it pressed in a cloth bag.

(vii) The rag from juice extracted by means of a screw-type juice extractor was heated for about 20 minutes with twice its weight of water and 0.3% citric acid and the extract strained through a piece of muslin cloth. (This extract was intensely bitter in taste).

Analytical methods. The soluble solids in the juice were determined by means of a refractometer and corrected to 20° C. Acidity of the juice was expressed as anhydrous citric acid by titration with standard N/10 NaOH solution. Ascorbic acid was determined by titration of the juice in 2% metaphosphoric acid solution with 2:6 dichlorophenol indophenol dye solution.

Preservation of juice. The juices were preserved by the overflow method by filling them into 12 oz. bottles at 80–83° C., closing with crown corks and pasteurizing for 25 minutes at 80° C. The bottles were stored at ordinary room temperature of 24–30° C. for about one year. Any off-flavour or bitterness in the juice in the different lots was recorded with the help of a taste panel of 12 judges selected from among the members of the staff of the Institute.

RESULTS AND DISCUSSION

In Table 1 are shown data regarding yield, physico-chemical composition, and organoleptic quality of the juice obtained by different methods of extraction. Data regarding the quality of the different juices bottled and examined at the end of a week's storage at room temperature (24–30° C.) are also given in the table. It will be noticed that the screw-type juice extractor gives the highest yield of juice (46.6%), while the lye-peeled segments and the juice sacs give only 34–39% of the juice. Squeezing by hand and pressing in a basket press give almost the same yield of juice (42–43%). The inner juice and outer juice together account for a yield of 40.4%. The ratio between the inner and outer juice is about 1:1, although it may vary in some cases from 1:1 to 3:1 depending upon size of fruit, size of the extraction cone, etc. Juice from lye-peeled segments has the lowest Brix. This is due to possible leaching out of some of the soluble solids in the segments during the lye-dipping and subsequent rinsing processes, which also are responsible for the lower yield of juice. The slight lowering of the acidity is due to partial neutralization of the juice by the alkali, and the slight decrease in ascorbic acid content is due to effect of heat and the alkaline medium, both of which are detrimental to the stability of ascorbic acid. The inner and outer juices have almost the same Brix, acidity and ascorbic acid content. There is very little loss of ascorbic acid of the juice when the juice is heated to 80° C. and cooled quickly. As regards the taste and flavour of the juice after bottling and keeping for a week, there are noticeable differences among the different treatments. The inner juice had very good taste and flavour, while the outer juice was almost equally good. Neither of them was bitter. The juice from the screw-type juice extractor was slightly bitter and so also the juice from the segments which were pressed in a basket press. The latter had a slight peculiar terpeny flavour also. The juice from the lye-dipped segments and also that from the separated juice sacs were free from any bitterness, but the former was slightly flat in taste.

Examination of the Preserved Juices After Long Storage

The different lots of bottled juice and extract were examined at the end of one year's storage at ordinary room temperature of 24–30° C. The data for the fresh juices and extracts used and the data for the bottled products at the end of the storage period are shown in Table 2. In the fresh state, the inner and outer juice as well as their combination and also the juice from the screw-type juice extractor were free from any marked bitterness. The residual juice expressed from the pulp and rag was not bitter, but had a slight raw taste. The water extract of the rag was, however, very bitter. Even the jelly made from it was bitter.

After storage, there was no bitterness in the inner or outer juice or their combination. The juice from the screw-type extractor had turned slightly bitter, while the residual juice squeezed from the rag was markedly bitter. Further, a cylindrical plug of jelly-like mass had separated in the bottles leaving a clarified liquid around it. The rag thus contains principles which are responsible not only for the development of bitterness in the pasteurized orange juice, but also for its gelation and clarification during storage. The negligible differences observed between the inner and outer juices as regards bitterness may be due to the fact that the pressure applied in the small device operated by hand might not have been sufficient to extract from the rind substances that are responsible for development of bitterness in the juice. The combined juice also was equally free from bitterness for the same reason. Thus, gentle extraction of the juice without incorporation of the extractable substances

TABLE 1

Effect of method of extraction on the yield, composition and quality of Coorg orange juice

Item No.	Sample	C.346 A	C.346 B	C.346 C	C.346 D	C.346 E	C.346 F	C.346 G
1	Method of juice extraction	Inner juice	Outer juice	Hand squeezing of segments in a cloth bag	Screw-type juice extractor	Segments lye dipped and pressed by hand in cloth	Juice sacs separated and pressed by hand in cloth	Segments crushed and then pressed in a basket press
2	Yield of juice %	21.1	19.3	43.4	46.6	33.6	38.5	42.1
3	Refractometer solids in juice (at 20° C.), %	13.0	13.0	13.5	12.0	8.5	11.5	11.0
4	Acidity of juice, as anhydrous citric acid, %	0.68	0.69	0.69	0.69	0.59	0.69	0.62
5	Ascorbic acid in fresh juice, mg./100 g.	27.1	28.6	31.0	21.1	16.5	25.7	21.0
6	Ascorbic acid in juice heated to 175° F. and cooled quickly, —mg./100 g.	26.4	26.4	31.1	21.1	14.9	24.5	21.2
7	Remarks (bottled juice)[1]	Clear with sediment; not bitter; a very good juice	Clear with sediment; not bitter; a good juice	Slightly cloudy, with sediment	Cloudy with much sediment; very slightly bitter	Clear with sediment; not bitter; slightly flat in taste	Clear with sediment; not bitter	Clear with sediment; slightly bitter; peculiar terpeny flavour

[1] Bottles were examined after one week's storage at room temperature of 24–30° C.

TABLE 2

Physico-chemical data for bottled Coorg orange juice, extracted by different methods, at the end of one year's storage at 24–30° C.

Item No.	Sample	C.351	C.351 A	C.351 B	C.351 C	C.351 D	C.351 E
1	Method of juice extraction	Inner juice	Outer juice	Inner juice and outer juice combined	Juice extracted with screw type juice extractor	Residual juice squeezed from pulp and rag of expt. C.351 and C.351/A	Water extract of rag and seed
2	Data for fresh juice taken[1]						
	i) Refractometer solids (at 20° C.), %	13.6	13.6	13.6	13.6	.....	7.0
	ii) Acidity as citric acid, %	0.62	0.60	0.60	0.60	.....	.....
	iii) Ascorbic acid, mg./100 g	22.8	21.2	22.4	27.3	(Not determined)	.....
	iv) Taste	Not bitter	Not bitter	Not bitter	Not bitter	Not bitter—slight raw taste	Very bitter (Jelly also made from it was bitter)
3	Data for bottled juice at the end of one year's storage						
	i) Refractometer solids (at 20° C.), %	13.6	14.6	14.6	14.1	14.1	.....
	ii) Acidity as citric acid, %	0.62	0.65	0.62	0.60	0.63	.....
	iii) Ascorbic acid, mg./100 g	14.5	15.1	14.8	20.3	19.9	.....
	iv) Retention of ascorbic acid, %	63.6	71.2	66.1	74.4	.....	.....
	v) Taste	Not bitter	Not bitter	Not bitter	Slightly bitter	Markedly bitter. Cylindrical plug of jelly had separated in the botle	Jelly also made from it was bitter

[1] The juices used were taken from a different lot of oranges as distinct from those for which data are given in Table 1.

from the rag and peel results in a juice that is free from bitterness.

As regards the retention of ascorbic acid during one year's storage, it is as high as 64–71% in the inner and outer juices and the combined juice. The retention in the juice extracted by means of a screw-type juice extractor is as high as 74% and the initial value for ascorbic acid content in this juice is also higher than that in the inner or outer juices.

SUMMARY

Gentle extraction of the juice from Coorg oranges helps in avoiding the incorporation of bitter constituents which are present mostly in the rag and peel of the fruit. Although the yield of juice can be increased by about 4–5% by heavy pressing, the resulting juice has a tendency to become slightly bitter. The extract of rag is intensely bitter, but the juice from the sacs only is free from any bitterness. Lye treatment of the segments removes bitterness, but the yield of juice is lowered slightly. The retention of ascorbic acid in bottled orange juice is as high as 65–75% during a storage period of one year at ordinary room temperature of 24–30° C. To avoid bitterness in the juice, the method of extraction should be such that very little of the rag or peel gets into the juice during extraction. The addition of 5–6% of sugar to the juice helps to mask any trace of bitterness that might still get into it.

Acknowledgment

The authors are most grateful to Dr. Girdhari Lal, Assistant Director, and Dr. V. Subrahmanyan, Director of the Institute, for their keen interest in this investigation.

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Effects of Gamma Ray Irradiation on Color and Chlorophyll Retention in Green Beans and Broccoli[a,b]

(Manuscript received May 31, 1958)

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THE DESTRUCTIVE EFFECT of ionizing radiations upon the color of green vegetables has been noted *(4)*; however, most of the reports of such radiation-induced "bleaching" of green vegetables appear to be based upon subjective visual observations.

The destruction of chlorophyll in a number of cathode ray-irradiated green vegetables was reported by Nickerson *et al. (8)*. They found that vacuum packing and irradiation in the frozen state were each effective in preventing the loss of chlorophyll due to irradiation.

In the present study, the degree of chlorophyll loss and color change in gamma-irradiated green beans and broccoli was determined. Various treatments were evaluated for their effectiveness in minimizing chlorophyll and color degradation.

MATERIALS AND METHODS

The various combinations of experimental conditions which were employed for each treatment are listed in Table 1. Ten cans of green beans and ten of broccoli were prepared for each treatment. Two cans out of the ten were used for analysis as soon as possible after irradiation, and two more were analyzed at the end of each of four subsequent storage periods.

Packing of whole green beans and broccoli. Green beans *(Phaseolus vulgaris)*, Valentine type, were cut into one-inch lengths with a commercial cutting machine, and blanched for 3 minutes in vigorously boiling water. After rapid cooling, 340 g. of beans were packed into each can. Except for the "dry" packs, 175 ml. of brine (1.5% NaCl) or water were added to the contents of each can. The "high-pH" samples were packed with 1.5% brine to which $MgCO_3$ (1.6 g./l.) had been added to give a pH of approximately 10. All samples were packed in No. 2 cans. R-enamel cans were used except for those treatments in which plain tin cans were specified.

After filling, air-packed samples were closed under atmospheric pressure. Vacuum-packed cans were sealed under 23 inches of vacuum using an American Can Co. Model No. 1 Pacific Semi-Automatic Can Closing Machine. In the case of nitrogen-packed cans, a vacuum of 22 inches was drawn and then released with nitrogen three times in succession. After closing, all cans were frozen in a room at −10° F.

Broccoli *(Brassica oleracea* var. *italica)*, Midway variety, was prepared for irradiation in essentially the same manner as described for green beans. The broccoli was cut longitudinally into sections averaging from four to five inches in length and packed into C-enamel cans. With both vegetables, the can lids were of the same type as the body of the can.

Irradiation of samples. All cans to be irradiated were packed in dry ice and shipped by air express in the frozen state to Argonne National Laboratory, Lemont, Illinois.

Cans of green beans and broccoli to be irradiated at room temperature were thawed just prior to irradiation. The cans whose contents were to be kept frozen during irradiation were removed at predetermined times in order to prevent thawing. After being cooled, these cans were re-immersed and the irradiation continued until the desired dose had been achieved. A dose of 1.86 megarads was given to all cans. After irradiation, the samples to be kept frozen were returned in dry ice by air express. The other samples were shipped at room temperature.

Time and temperature of storage. Control samples, both irradiated and non-irradiated were stored at 0° F. All other samples were stored either at room temperature (ca. 75° F.) or at 100° F. The initial green bean color and pigment determinations were carried out 3 weeks after irradiation. Subsequent determinations were made after post-irradiation storage periods of 7, 15, 25, and 48 weeks. In the case of broccoli, the initial determination were performed 2 weeks after irradiation. Subsequent determinations were made after post-irradiation storage periods of 6, 16, 25, and 46 weeks.

Sealed tube studies with puréed green beans and broccoli. The raw material for these studies consisted of the above mentioned broccoli and green beans which were cut, blanched, sealed in bulk in large plastic bags, and stored at 0° F. for 11 months prior to use.

Each vegetable was puréed by passage through a Fitzpatrick Comminuting Machine (Model M.).[e] Following the addition of water, ascorbic acid solution, or buffer solution to each portion of purée, as described below, the material was deaerated. Pyrex tubes (2 x 10 cm.) were filled with purée, and the tops of the tubes were drawn into capillaries. Air remaining in the headspace of the tubes was removed by repeated evacuations followed by nitrogen release, after which the capillaries were sealed.

The sealed tubes were rapidly frozen, and placed in No. 2 cans along with adequate cushioning material to prevent breakage. Cans were sealed and packed in dry ice for shipment to the Argonne National Laboratory. All samples were thawed just prior to irradiation and were irradiated at room temperature.

The pH values of a series of puréed green bean and broccoli samples were elevated by the addition of $NaOH–KH_2PO_4$ buffer. After pH adjustment, tubes were filled, sealed, and irradiated in the manner already described. All samples received a dose of 1.86 megarads.

Magnesium (as $MgCO_3$) was added to some of the puréed

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[a] Contribution No. 1156, University of Massachusetts College of Agriculture Experiment Station. This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM R&E Command, U. S. Army, and has been assigned number 923 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsements of the Department of Defense.

[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, Illinois, May 28, 1958.

[c] Present address: Eastman Chemical Products, Inc., Kingsport, Tennessee.

[d] Present address: Continental Baking Co., Rye, N. Y.

[e] Manufactured by the W. J. Fitzpatrick Co., 1001 W. Washington Blvd., Chicago 7, Illinois.

TABLE 1

Experimental treatments for green beans and broccoli

Treatment No.	Irrad. temp.	pH	Can lining	Packing medium	Headspace gas	Storage temp.
1	Room temp.	Normal	Plain tin	Brine	Vacuum	Room temp.
2	Room temp.	High	Plain tin	Brine	Vacuum	Room temp.
3	Room temp.	Normal	Enameled	Brine	Vacuum	Room temp.
4	Room temp.	High	Enameled	Brine	Vacuum	Room temp.
5	Room temp.	Normal	Enameled	Brine	Nitrogen	Room temp.
6	Room temp.	Normal	Enameled	Water	Vacuum	Room temp.
7	Room temp.	Normal	Enameled	Dry	Vacuum	Room temp.
8	Room temp.	Normal	Enameled	Dry	Nitrogen	Room temp.
9	Room temp.	Normal	Enameled	Dry	Air	Room temp.
10	Room temp.	Normal	Enameled	Brine	Vacuum	100° F.
11	Frozen	Normal	Plain tin	Brine	Vacuum	Room temp.
12	Frozen	High	Plain tin	Brine	Vacuum	Room temp.
13	Frozen	Normal	Enameled	Brine	Vacuum	Room temp.
14	Frozen	High	Enameled	Brine	Vacuum	Room temp.
15	Frozen	Normal	Enameled	Brine	Nitrogen	Room temp.
16	Frozen	Normal	Enameled	Water	Vacuum	Room temp.
17	Frozen	Normal	Enameled	Dry	Vacuum	Room temp.
18	Frozen	Normal	Enameled	Dry	Nitrogen	Room temp.
19	Frozen	Normal	Enameled	Dry	Air	Room temp.
20	Frozen	Normal	Enameled	Brine	Vacuum	100° F.
21	Frozen	Normal	Enameled	Brine	Vacuum	0° F.
22	Non-irrad. control	Normal	Enameled	Brine	Vacuum	0° F.

broccoli samples along with the buffer. Purées prepared in this manner contained approximately three times as much added magnesium as was naturally present in the chlorophyll of the purée. These samples also received a dose of 1.86 megarads.

A series of green bean and broccoli purées containing the following levels of added ascorbic acid was prepared: 0.01%, 0.05%, 0.10%, and 0.50%. Ascorbic acid is expressed in terms of per cent (by weight) in the diluted purée. All samples received a dose of 1.86 megarads.

A series of replicate tubes containing green bean purée, and another series containing broccoli purée were prepared. Samples of each vegetable were irradiated with the following doses: 0.49, 0.87, 1.96, 3.65, 5.49 and 7.29 megarads.

Measurement of chlorophyll. Can contents were put through a meat grinder in order to facilitate representative sampling. A weighed amount (ranging from 5 to 10 g.) was transferred to a medium porosity fritted glass buchner (150 ml. capacity). Successive small volumes of 85% acetone were added to the funnel, stirred thoroughly with the sample, and then filtered with suction. Extraction was continued until color was no longer evident in the last volume of filtrate. Duplicate samples from each can were extracted in this manner.

The extract was then re-filtered through a fine porosity fritted glass buchner funnel (60 ml. capacity) directly into a 100 ml. volumetric flask, and made up to 100 ml. with 80% acetone. Optical density measurements were made at 663 mμ and 645 mμ with a Beckman model DU quartz spectrophotometer.[f]

Calculations were based upon specific absorption coefficients for chlorophyll given by Mackinney *(5)*, using equations set up by Arnon *(1)* and modified to yield mg. chlorophyll/100 g. of dry material. The necessary moisture determinations were carried out by drying for 8 hours in a vacuum oven at 70° C.

Measurement of chlorophyll conversion to pheophytin. Extracts which were prepared for the determination of chlorophyll content were also used for pheophytin measurement. Optical density measurements were made at 560 mμ and 535 mμ, as described in the method of Mackinney and Weast *(6)*.

Reflectance measurements. The surface color of green bean samples was measured with a Gardner Color[g] Difference Meter (formerly known as the Hunter Color and Color Difference Meter). The Gardner instrument was set up in the manner described by Francis *(3)*.

In the case of broccoli, samples were ground before taking reflectance measurements. This was necessary because of the non-uniformity of the broccoli, especially between stem and flower portions. Gardner measurements of the ground product were made in circular glass cells (2¼ in. inner diameter) having optical glass bottoms.

All measurements taken with the Gardner instrument were made with large area illumination, except in the case of the sealed tube purée experiments. The small amounts of sample available in the purée experiments made it necessary to use small area illumination, and also necessitated the use of small Plexiglas cells (1 inch diameter) as sample holders.

Gardner measurements of Rd, "a", and "b" were made using a standard porcelain tile having the following values: Rd = 8.8; a = −0.6; b = +19.7. The calculated $\tan^{-1}a/b$ *(8)* value was used as an index of hue, while $(a^2 + b^2)^{1/2}$ served as a measure of chroma. Brightness was indicated by the Rd value.

pH measurements. The pH value of all samples were measured with a Beckman model G pH meter.

Statistical analysis. Significance of differences between treatments was tested by the analysis of variance, using the 1% level of significance.[h]

RESULTS AND DISCUSSION

Overall effects of irradiation and storage. Gamma-irradiation of green beans in cans with a 1.86 megarad dose resulted in approximately 40% destruction of the original chlorophyll content, while with broccoli, the destruction amounted to approximately 28%. The degree of destruction varied widely, depending upon the particular treatment. The figures given above are averages for treatments 1–20 (listed in Table 1) for each vegetable at the time of the first analysis after irradiation. During storage after irradiation there was little further decrease in chlorophyll content of either vegetable (Table 2).

Both broccoli and green beans became markedly yellower as a result of irradiation. In the case of green beans, post-irradiation storage produced a significant hue change in the direction of a greener color. Though statistically significant, the improvement in hue during storage was of much smaller magnitude than the hue change which took place during irradiation. Broccoli also shifted in hue toward the green

[f] Manufactured by Beckman Instrument, Inc., 2500 Fullerton Road, Fullerton, Calif.

[g] Manufactured by Gardner Laboratory, Inc., P. P. B. 5728, Bethesda 14, Md.

[h] In the interests of brevity, the tables of analyses of variance have been omitted. They will be supplied by the authors upon request.

TABLE 2

Effect of irradiation temperature upon chlorophyll content and hue of green beans and broccoli stored at room temperature

Post-irradiation Storage weeks	Chlorophyll		Hue	
	Irrad. room temp.	Irrad. frozen	Irrad. room temp.	Irrad. frozen
	(mg./100 g. dry wt.)	(mg./100 g. dry wt.)	$\tan^{-1}a/b$	$\tan^{-1}a/b$
Green Beans				
Before irradiation..	130[1]	130	−28.1	−28.1
3	77	91	−2.0	−5.0
7	79	90	−4.8	−7.5
12	78	95	−4.0	−7.0
24	74	82	−5.0	−6.9
48	77	86	−7.6	−9.8
Broccoli				
Before irradiation..	155	155	−22.4	−22.4
2	116	133	−7.1	−7.6
6	120	127	−4.9	−6.1
16	117	126	−4.5	−5.7
25	102	112	−3.2	−5.4
46	103	124	−6.5	−9.7

[1] Each datum was obtained from an average of analyses on each of 20 cans, 2 for each treatment described in Table 1.

during the latter part of storage, but this was preceded by a change toward the yellow during earlier stages of storage (Table 3).

Both vegetables showed slight decreases in chroma as a result of irradiation. During storage, the chromas of many of the samples changed significantly between storage periods, but the changes were too erratic to indicate a definite trend. The overall effect of storage for most treatments was a slight increase in chroma.

An increase in pheophytin content resulted from irradiation of both green beans and broccoli. Comparatively low pheophytin values at the time of the initial analysis were noted only in the high-pH group of samples. During storage, most samples showed an increase in pheophytin content for a period of time followed by a decrease during the latter part of storage. The decrease in pheophytin content would seem to indicate that pheophytin, produced by the degradation of chlorophyll, was itself being broken down. It should be pointed out, however, that many of the calculated pheophytin values for both vegetables were over 100%. The most probable explanation for such high values is that one or more interfering substances were present in the pigment extracts, and contributed to the total absorption at the wavelengths used for pheophytin measurement.

TABLE 3

Effect of pH adjustment upon chlorophyll retention and hue in gamma-irradiated green beans and broccoli stored at room temperature

Post-irradiation Storage weeks	Chlorophyll		Hue	
	Normal pH	High pH	Normal pH	High pH
	(mg./100 g. dry wt.)	(mg./100 g. dry wt.)	$\tan^{-1}a/b$	$\tan^{-1}a/b$
Green Beans				
Before irradiation.....	130[1]	130	−28.1	−28.1
3	87	102	−5.3	−20.0
7	82	82	−3.9	−7.5
15	90	85	−4.9	−6.0
25	82	77	−5.3	−6.2
48	83	77	−5.4	−7.5
Broccoli				
Before irradiation.....	155	155	−22.4	−22.4
2	118	146	−7.9	−23.8
6	118	132	−5.3	−12.0
16	116	133	−4.6	−9.4
25	112	109	−4.0	−7.6
46	107	123	−9.8	−13.6

[1] Each datum was obtained from an average of analyses on each of 8 cans, 2 for each of treatments 1 to 4 and 11 to 14 of Table 1.

Effect of pH adjustment. Raising the pH of the brine in which broccoli and green beans were packed prevented much of the chlorophyll destruction and loss of green color that took place during irradiation of samples packed in normal-pH brine. However, during post-irradiation storage of both vegetables, greater chlorophyll destruction and loss of greenness occurred in the high-pH group of samples than in the group to which no alkalizing agent had been added. In spite of the greater destruction, the broccoli samples with high pH values were significantly greener and higher in chlorophyll content than their normal-pH counterparts, although the magnitude of the difference between the 2 groups was diminished with increasing storage time (Table 4). The chlorophyll content of green beans, by the end of the seventh week of storage, was no greater in the high-pH samples than in those packed at normal-pH. During continued storage, the chlorophyll content of the high-pH group was even lower than that of the samples packed with normal-pH brine. The initial difference between the $\tan^{-1}a/b$ values of green beans packed with and without added alkalizing agent had largely disappeared by the seventh week of storage. Although the high-pH samples were greener throughout storage, the difference in hue was not significant during much of the latter part of storage (Table 4).

Just after irradiation and during the early part of storage, the high-pH groups of broccoli and green bean samples were lower in pheophytin than were the corresponding normal-pH groups. During the latter part of storage, neither of the vegetables exhibited a significant difference in pheophytin due to the use of high-pH brine.

High-pH broccoli samples were brighter than were the samples in the normal-pH group, but brightness of irradiated green beans was not altered by pH adjustment. There was no significant effect of pH adjustment on the chroma of broccoli samples, but some of the green bean samples whose pH had been raised were significantly higher in chroma.

During the storage of irradiated green beans, the pH of samples with added alkalizing agent decreased from pH 7.2 to pH 5.5, while the normal-pH group dropped from pH 5.9 to pH 5.3. Broccoli samples with added alkalizing agent decreased in pH from an initial value of pH 7.1 to pH 5.9 by the end of storage, while normal-pH broccoli samples showed a pH decrease from pH 6.0 to pH 5.4. With both vegetables, the rate of decrease in pH was highest during the early part of storage.

Since the precipitous drop in chlorophyll content and the sharp change in hue of high-pH samples shortly after irradiation was accompanied by a relatively large decrease in pH, the relationship between pH, color and chlorophyll content was explored further in experiments using purées. Data are shown in Table 4 for chlorophyll content and color of irradi-

TABLE 4

Effect of pH upon chlorophyll retention and color of irradiated green bean and broccoli purées[1]

pH		Chlorophyll[2] (mg./100 g. dry)	Color measurements		
Before irradiation	After irradiation		Rd	$\tan^{-1}a/b$	$(a^2+b^2)^{1/2}$
Green Bean Purée					
6.0[3]	....	109	9.9	−16.3	15.1
6.0	6.0	75	5.9	−17.5	12.5
6.1	6.1	58	7.0	−17.5	13.6
6.4	6.4	58	6.5	−14.5	13.6
6.7	6.7	58	5.5	−17.2	11.8
7.6	7.6	41	4.6	−21.9	12.0
8.2	8.0	54	4.4	−21.7	11.1
Broccoli Purée					
6.0[3]	....	116	10.8	−17.0	19.6
6.0	5.9	81	7.8	−12.8	16.2
6.2	6.1	60	8.2	−17.4	17.1
6.2[4]	6.1	58	8.3	−16.6	16.8
6.6	6.5	60	7.3	−22.9	16.7
6.8	6.7	60	6.6	−22.3	16.4
7.4	7.3	68	5.6	−16.2	14.4
7.4[4]	7.3	60	5.6	−15.8	14.4
8.1	7.7	66	4.9	−14.4	13.7
8.1[4]	7.7	59	4.9	−13.6	13.7
8.4	7.9	81	4.9	−15.2	13.8
9.0	8.0	76	5.2	−12.7	14.0

[1] All samples received a gamma radiation dose of 1.86 megarads.
[2] Each chlorophyll value represents the average of duplicate determinations.
[3] Non-irradiated control.
[4] Magnesium (as $MgCO_3$) added in addition to buffer during preparation of these purées.

ated broccoli and green bean purées, buffered at various pH levels. In general, the beneficial effect of pH adjustment upon chlorophyll retention previously noted with the cut vegetables was not reproduced among the irradiated purée samples.

At most of the elevated pH levels, purée samples were somewhat greener than controls which had been irradiated without prior pH adjustment. The improvement in hue of broccoli purée was most pronounced at pH 6.6 and pH 6.8, while with green bean purée, pH 7.6 and pH 8.2 gave the greenest color.

Effect of added magnesium. The addition of magnesium (as $MgCO_3$) to broccoli purée had no effect on chlorophyll retention, brightness, or chroma, as shown by the data in Table 4. Hue was slightly greener in samples without the added magnesium.

Effect of temperature of product during irradiation. When kept frozen during irradiation, both green beans and broccoli exhibited significantly greater chlorophyll retention and were significantly greener than when irradiated while unfrozen, as shown in Table 2. There were only a few treatments (e.g.—the air-packed green bean samples, treatments No. 9 and No. 19) where the effect was not significant. Irradiation in the frozen state tended to result in increased brightness of both vegetables. The chroma of broccoli samples which were irradiated while frozen was generally higher than that of the comparable room temperature-irradiated group, but in green beans the effect on chroma tended to be in the opposite direction. The pheophytin content of green beans which were irradiated while frozen was usually higher than in the samples irradiated at room temperature. With a majority of the broccoli samples, however, there was no significant effect of irradiation temperature on pheophytin content.

Effect of headspace gas. Green beans which were packed dry with air as the headspace gas showed significantly lower chlorophyll retention than the comparable nitrogen- and vacuum-packed samples (Table 5). With broccoli, however, irradiation and storage of air-packed samples resulted in significantly greater chlorophyll retention than did nitrogen- or vacuum-packing.

When compared with nitrogen-packed samples, the use of air as the headspace gas produced a greener hue in the case of broccoli, and had the opposite effect on the hue of green beans. There was no significant difference in hue between the air-packed and vacuum-packed groups of either vegetable.

Use of air, as compared with nitrogen or vacuum produced significantly brighter green beans but in the case of broccoli tended to result in a darker product. Chroma of air-packed broccoli was significantly lower but the effect on chroma of green beans varied considerably depending upon the particular treatment.

The pheophytin content of both vegetables was significantly lower as a result of air-packing.

A comparison of samples packed dry under vacuum or nitrogen (Table 5) showed that vacuum-packing of broccoli generally resulted in a greener product, while the opposite effect was noted in most of the green bean samples. The other significant differences which were observed in green beans in some instances were higher chlorophyll, pheophytin and chroma values in the vacuum-packed groups. The substitution of nitrogen for vacuum in the headspace of samples packed in brine did not introduce any major differences (Tables 6 and 7). Vacuum packing of broccoli resulted in slightly greener hues, but the green beans were yellower when irradiated at room temperature and much greener when irradiated frozen.

Effect of added ascorbic acid. Although there was some indication of increased chlorophyll retention in irradiated green bean and broccoli purées to which

TABLE 5

The color and chlorophyll content of green beans and broccoli packed dry in enameled cans and stored at room temperature for twelve months

	Irrad. at room temp.			Irrad. frozen		
	Vacuum	Nitrogen	Air	Vacuum	Nitrogen	Air
Green Beans						
Chlorophyll, (mg./100 g.)	83[1]	77	70	93	78	65
Reflectance (Rd)	7.7	7.9	8.6	7.7	7.6	9.6
Hue ($\tan^{-1}a/b$)	−4.6	−6.0	−4.8	−4.7	−8.7	−4.3
Chroma $(a^2+b^2)^{1/2}$	12.3	11.4	11.2	12.5	10.6	12.2
Broccoli						
Chlorophyll, (mg./100 g.)	103	99	114	119	122	129
Reflectance (Rd)	9.6	9.8	9.1	10.1	9.3	8.2
Hue ($\tan^{-1}a/b$)	−5.2	−3.8	−5.2	−6.4	−4.3	−7.2
Chroma $(a^2+b^2)^{1/2}$	16.7	16.8	15.8	17.1	17.3	16.3

[1] Each datum was obtained from an average of 10 cans, i.e., 2 at each of 5 storage periods.

TABLE 6

The color and chlorophyll content of green beans and broccoli packed under vacuum in enameled cans and stored at room temperature for twelve months

	Irrad. at room temp.		Irrad. frozen	
	Brine	Water	Brine	Water
Green Beans				
Chlorophyll (mg./100 g.)	76[1]	80	88	102
Reflectance (Rd)	7.0	7.0	7.6	8.0
Hue ($\tan^{-1}a/b$)	−2.2	−4.4	−7.8	−4.1
Chroma $(a^2 + b^2)^{1/2}$	11.6	11.4	10.5	12.5
Broccoli				
Chlorophyll (mg./100 g.)	109	111	114	123
Reflectance (Rd)	11.3	11.1	11.6	12.0
Hue ($\tan^{-1}a/b$)	−5.4	−3.6	−5.4	−5.2
Chroma $(a^2 + b^2)^{1/2}$	16.5	16.9	18.2	17.8

[1] Each datum was obtained from an average of 10 cans, i.e., 2 at each of 5 storage periods.

TABLE 7

The color and chlorophyll content of green beans and broccoli packed in brine and stored at room temperature for twelve months

	Plain tin cans		Enameled cans	
	Vacuum headspace		Nitrogen headspace[1]	
	Irrad. at R.T.	Irrad. frozen	Irrad. at R.T.	Irrad. frozen
Green Beans				
Chlorophyll (mg./100 g.)	77[2]	98	72	92
Reflectance (Rd)	8.6	9.1	7.3	7.7
Hue ($\tan^{-1}a/b$)	−2.7	−4.0	−4.3	−6.9
Chroma $(a^2 + b^2)^{1/2}$	14.2	14.6	11.4	10.7
Broccoli				
Chlorophyll (mg./100 g.)	113	121	109	121
Reflectance (Rd)	11.0	11.9	10.5	11.8
Hue ($\tan^{-1}a/b$)	−3.3	−4.8	−3.5	−5.0
Chroma $(a^2 + b^2)^{1/2}$	17.7	18.2	18.0	17.9

[1] Data for comparable cans with a vacuum headspace is presented in Table 6.

[2] Each datum was obtained from an average of 10 cans; i.e., 2 at each of 5 storage periods.

ascorbic acid had been added, the results were inconclusive. Under the conditions of the experiment, the protective effect of added ascorbic acid appeared to be negligible.

Effect of packing medium. When prepared for irradiation without added liquid, irradiated green beans tended to be greener and higher in chlorophyll content than the comparable brine-packed samples (Tables 5 and 6). Hue and chlorophyll content of broccoli samples were not affected by the absence of brine. Most of the broccoli samples were, however, significantly darker and lower in chroma when packed "dry." Pheophytin content of both vegetables was generally unaffected by the change in packing medium.

Substitution of water for brine as the packing medium for broccoli resulted in a yellower hue and no significant change in chlorophyll content (Table 6). With green beans, water-packed samples were higher in chlorophyll content, while the hue of water-packed green beans was significantly greener for some of the samples and yellower for the remainder.

Effect of can lining. In approximately half of the samples studied, the substitution of plain tin for enameled cans did not significantly affect the hue of irradiated green beans or broccoli. In the remainder of the samples, a significantly greener color was achieved when enameled cans were used. Chlorophyll retention in green beans was significantly higher in the plain tin-packed samples, but no difference in the chlorophyll content of broccoli resulted from the variation in can lining.

The most striking effects of can lining were observed in the significantly greater brightness and chroma of green beans packed in plain tin rather than enameled cans (Table 7). Brightness and chroma of some of the broccoli samples were also significantly higher due to the use of plain tin cans. The pheophytin content of broccoli was unaffected by can lining, whereas in some of the green bean samples, the use of plain tin cans resulted in a significantly higher pheophytin content.

Effect of storage temperature. Storage at 100° F. rather than at room temperature did not affect the chlorophyll content or hue of irradiated green beans. With broccoli, however, both color and chlorophyll retention were better in samples stored at the higher temperature, although the improvement in hue resulting from 100° F. storage was not evident during the early part of storage (Table 8).

Optimum conditions for minimizing chlorophyll and color degradation. Pigment content and color for 10 treatments which appeared to be the most desirable from a pigment and color point of view are presented in Table 9. Treatments were arranged in order for the various attributes of color as it was assumed that a higher hue value indicating a greener color, a higher Rd value which indicates a brighter product and a higher chroma indicating a more intense color, would be desirable. This assumption should be the subject of further research.

Color and pigment values for the irradiated and non-irradiated samples stored at zero ° F. are also presented in order that the irradiated samples could be compared with the frozen controls. It will be seen that there was considerable change even with the optimum conditions. The irradiated samples were somewhat inferior to the fresh or frozen samples on the market.

The choice of the optimum conditions for irradiation would have to be governed by the weighting

TABLE 8

The effect of storage temperature on the color and chlorophyll content of green beans and broccoli packed in brine under vacuum in enameled cans

	Stored at 100° F.[1]		Stored at 0° F.
	Irrad. at R.T.	Irrad. frozen	Irrad. frozen
Green Beans			
Chlorophyll (mg./100 g.)	80[2]	89	130
Reflectance (Rd)	6.9	8.0	7.8
Hue ($\tan^{-1}a/b$)	−4.0	−8.1	−25.2
Chroma $(a^2 + b^2)^{1/2}$	11.4	11.3	14.8
Broccoli			
Chlorophyll (mg./100 g.)	119	125	168
Reflectance (Rd)	10.0	11.0	11.8
Hue ($\tan^{-1}a/b$)	−4.9	−7.2	−24.6
Chroma $(a^2 + b^2)^{1/2}$	16.4	17.6	20.1

[1] Data for comparable cans stored at ca. 70° F. is presented in Table 6.

[2] Each datum was obtained from an average of 10 cans; i.e., 2 at each of 5 storage periods.

TABLE 9

Ten treatments which least affect the color and chlorophyll content of green beans and broccoli subjected to irradiation

Chlorophyll mg./100 g. dry wt.	Hue $\tan^{-1}a/b$	Reflectance Rd	Chroma $(a^2+b^2)^{1/2}$
Green Beans			
102[1] (16)[2]	−13.4 (14)	9.6 (19)	15.3 (12)
98 (11)	− 8.7 (18)	9.3 (12)	14.6 (2)
95 (12)	− 8.6 (12)	9.1 (11)	14.6 (11)
93 (17)	− 8.1 (20)	8.6 (1)	12.5 (17)
92 (15)	− 7.8 (13)	8.6 (9)	12.5 (16)
89 (20)	− 6.9 (15)	8.4 (2)	12.3 (7)
88 (14)	− 6.5 (4)	8.0 (14)	12.2 (19)
88 (13)	− 6.0 (8)	8.0 (20)	11.6 (3)
83 (7)	− 5.7 (2)	8.0 (16)	11.4 (5)
80 (6)	− 4.7 (17)	7.9 (8)	11.4 (6)
130[3] (21)	−25.2 (21)	7.8 (21)	14.8 (21)
130 (22)	−28.8 (22)	8.4 (22)	16.9 (22)
Broccoli			
138 (14)	−11.6 (12)	12.0 (16)	18.6 (14)
135 (12)	−10.5 (14)	11.9 (11)	18.3 (12)
129 (19)	− 9.0 (4)	11.8 (15)	18.2 (13)
125 (20)	− 8.6 (2)	11.7 (12)	18.2 (11)
123 (16)	− 7.2 (19)	11.6 (13)	18.0 (5)
122 (18)	− 7.2 (20)	11.2 (3)	17.9 (15)
121 (15)	− 6.4 (17)	11.1 (6)	17.8 (16)
121 (11)	− 5.4 (3)	11.1 (14)	17.7 (1)
121 (4)	− 5.2 (9)	11.0 (20)	17.6 (20)
121 (2)	− 5.2 (16)	11.0 (1)	17.5 (2)
168 (21)	−24.6 (21)	11.8 (21)	20.1 (21)
155 (22)	−22.3 (22)	12.1 (22)	22.0 (22)

[1] Each datum represents the average obtained from 10 cans, i.e., duplicate analyses at each of five storage periods.

[2] The figures in brackets represent the treatment numbers as described in Table 1.

[3] Treatment No's. 21 and 22 represent the irradiated and non-irradiated samples stored at zero °F.

given to a number of quality attributes. From a color and pigment point of view, and equal weighting for the attributes in Table 9, it would appear that Treatment No. 12 would be optimum for both green beans and broccoli. This would involve packing the product in a high pH brine under vacuum in plain tin cans and irradiating in the frozen state. For long storage periods, packing in enamel cans (Treatment No. 14) might be desirable.

Use of higher than 1.86 megarad levels of gamma radiation to achieve sterilization has been suggested by Morgan *(7)*. The relationship between chlorophyll retention and irradiation dose, shown in Table 10, would indicate that the chlorophyll degradation at higher doses would be greater than that observed at the 1.86 megarad levels employed in this study.

TABLE 10

Effect of dose of gamma-irradiation at room temperature upon chlorophyll retention in green bean and broccoli purées[1]

Irradiation dose, megarads	Per cent retention of chlorophyll	
	Green beans	Broccoli
0.5	98	98
0.9	91	91
2.0	77	82
3.7	62	63
5.5	59	61
7.3	37	31

[1] The purées in this experiment were irradiated in ampoules as indicated in the methods.

SUMMARY AND CONCLUSIONS

The effects of gamma radiation upon color and pigment content of green beans and broccoli have been described.

With a 1.86 megarad dose, a marked change in hue from the green toward the yellow took place in both vegetables. Irradiation produced a decrease in chlorophyll content and an increase in pheophytin. In the case of green beans, the destruction of chlorophyll amounted to about 40 per cent of the amount originally present. With broccoli, approximately 28 per cent of the chlorophyll content of the non-irradiated vegetables was destroyed. There was little, if any, further decrease in the chlorophyll content of either vegetable during post-irradiation storage, except for samples whose pH had been raised. A decrease in the pheophytin content of both vegetables took place during the latter part of storage.

Green beans and broccoli were slightly greener after one year's storage. Irradiation in the frozen state produced brighter and greener samples with a higher chlorophyll content. Packing green beans and broccoli in brine with a higher pH value prevented much of the chlorophyll breakdown and color loss. This improvement was retained to some extent with broccoli upon storage but not with green beans.

The use of plain tin instead of enameled cans, under certain conditions, resulted in poorer color retention in both broccoli and green beans. However, chlorophyll content was higher in irradiated green beans which were packed in plain tin cans. Green beans packed under vacuum or nitrogen showed increased chlorophyll retention and less yellowing than the air packed samples. The reverse was true for broccoli.

The combination of conditions which provided the greatest degree of protection against change in color and chlorophyll content consisted of immersion in high-pH brine, vacuum-packing in plain tin cans, and irradiation in the frozen state.

A linear decrease in chlorophyll content of both green beans and broccoli resulted from increases in gamma radiation dose, over the range of 0.49 to 7.29 megarads.

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Effects of Gamma Ray Irradiation on Carotenoid Retention and Color of Carrots, Sweet Potatoes, Green Beans and Broccoli [a,b]

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A NUMBER OF OBSERVERS have reported that irradiation caused either a visible bleaching or a darkening effect on vegetables *(3)*. Huber *(4)* used a Capacitron electron accelerator to produce sterilizing dosages and reported considerable bleaching of carrots and some bleaching of green beans. Lukton and Mackinney *(5)* studied the breakdown of carotenoids by irradiation in a number of products. They concluded that "the destruction of carotenoid pigments on exposure to gamma-radiation is caused by secondary reactions and depends upon the extent to which free radicals or peroxides formed in the surrounding medium are available for reaction."

The present study was concerned with the retention of carotene and xanthophyll in green beans and broccoli; color, total carotenoids, and beta-carotene and xanthophyll in sweet potatoes; and color total carotenoids, beta-carotene, alpha-carotene and xanthophyll in carrots when irradiated under a variety of conditions.

MATERIALS AND METHODS

Preparation of samples. Green beans and broccoli were prepared as described by Wishnetsky *(9)*. The same samples were used for this work and the treatments are listed in Table 1.

Maryland Golden variety sweet potatoes were heated for 5 minutes at 240° F. to loosen the skins which were removed under cold water. The peeled potatoes were cut into chunks, packed, sealed and frozen according the methods described for green beans and broccoli. The sweet potatoes were packed in R-enamel or plain tin cans with a 30% (wt./vol.) sucrose syrup where indicated in Table 1.

The sweet potatoes used for the enzyme studies reported in Table 2 were heated at 240° F. to loosen the skins and peeled under cold water. Where blanching was indicated in Table 2, the potatoes were sliced and heated in steam to 212° F. for 5 minutes. After cooling, the slices were packed in plain tin No. 2 cans in 30% (wt./vol.) sucrose syrup, sealed under vacuum and immediately frozen. The unblanched samples were sliced or puréed after peeling and handled in the same manner as the sliced samples except that no sugar syrup was added.

The samples in glass vials (approximately 2 x 8 cm.) were packed by placing 10 g. in each vial and stoppering the vials under 23 inches of vacuum in a White Cap vacuum sealer. Two vials were packed in cotton in each No. 2 can.

The slices with potassium cyanide, which was added to inhibit enzyme action, were dipped in a 4% solution of potassium cyanide for 25 minutes prior to packing in syrup. The puréed samples had sufficient cyanide added to make the concentration 10,000 p.p.m. in the purée. The samples with chlorogenic acid had 1400 p.p.m. in the purée.

The sliced or puréed samples of sweet potatoes were irradiated in the frozen state with 1.86 megarads [e] and stored for 2, 6, 15, and 22 weeks at room temperature.

Imperator type carrots were used for the treatments listed in Table 1. They were prepared in essentially the same manner as described for sweet potatoes except that they were sliced rather than cut into chunks and a 1.5% sodium chloride brine was added rather than syrup.

Analytical methods. For green beans and broccoli, a suitable aliquot of the acetone extract prepared for chlorophyll analysis, was transferred to hexane in a separatory funnel. After washing, the hexane layer was saponified with 20% methanolic potassium hydroxide solution in the manner described by Zscheile and Porter *(10)*. The hexane portion was extracted with 90% methyl alcohol in water, washed with water and made up to volume. The carotene solution in the hexane was read in an Evelyn colorimeter equipped with a 440 mμ filter and calibrated with beta-carotene purified from 100 per cent beta-carotene (Eastman Kodak) according to the method outlined in the *Official Methods of Analysis* of the A.O.A.C. (8th. edit., 1955). The methanolic potassium hydroxide and methanol in water solutions containing the xanthophylls were combined in a separatory funnel. The xanthophyll pigments were transferred to hexane by diluting with water and washed to remove traces of methanol. The hexane solution was made to volume and read in an Evelyn [f] colorimeter with a 440 mμ filter and calculated as β-carotene.

In the case of sweet potatoes, the xanthophyll, β-carotene and total carotenoids were determined by the method of Wall and Kelly *(8)*. The carotenoids were extracted with a mixture of ethanol and hexane and transferred to hexane by the addition of water. The xanthophylls were removed by extracting with alcoholic potassium hydroxide solution. The hexane solution, after washing with water, was made to volume and read as an index of total carotenoids. An aliquot of the hexane solution was chromatographed on a 1:1 MgO-Celite [g] column to separate the β-carotene. The hexane solution was washed 5 times with water before chromatographing, to remove traces of ethanol which interfered with the development of the column. The xanthophyll solution was transferred to hexane, washed, and made to volume. All pigment solutions were read in an Evelyn colorimeter at 440 mμ, calibrated for β-carotene.

The pigment content of carrots was determined in the same manner as that of sweet potatoes except that α-carotene was also separated on the MgO-Celite column. The xanthophyll,

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[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, Illinois, May 28, 1958.

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[e] At the Argonne National Laboratories, Lemont, Ill.

[f] Manufactured by the Rubicon Co., Div. of Minneapolis Honeywell, Philadelphia, Pa.

[g] Obtained from the Johns-Manville Co., Baltimore, Md.

TABLE 1

Experimental treatments for green beans, broccoli, sweet potatoes and carrots

Treatment No.	Irrad. temp.	pH	Can lining	Packing medium	Headspace gas	Storage temp.
1	Room temp.	Normal	Plain tin	Brine	Vacuum	Room temp.
2[1]	Room temp.	High	Plain tin	Brine	Vacuum	Room temp.
3	Room temp.	Normal	Enameled	Brine	Vacuum	Room temp.
4[1]	Room temp.	High	Enameled	Brine	Vacuum	Room temp.
5	Room temp.	Normal	Enameled	Brine	Nitrogen	Room temp.
6	Room temp.	Normal	Enameled	Water	Vacuum	Room temp.
7	Room temp.	Normal	Enameled	Dry	Vacuum	Room temp.
8	Room temp.	Normal	Enameled	Dry	Nitrogen	Room temp.
9	Room temp.	Normal	Enameled	Dry	Air	Room temp.
10	Room temp.	Normal	Enameled	Brine	Vacuum	100° F.
11	Frozen	Normal	Plain tin	Brine	Vacuum	Room temp.
12[1]	Frozen	High	Plain tin	Brine	Vacuum	Room temp.
13	Frozen	Normal	Enameled	Brine	Vacuum	Room temp.
14[1]	Frozen	High	Enameled	Brine	Vacuum	Room temp.
15	Frozen	Normal	Enameled	Brine	Nitrogen	Room temp.
16	Frozen	Normal	Enameled	Water	Vacuum	Room temp.
17	Frozen	Normal	Enameled	Dry	Vacuum	Room temp.
18	Frozen	Normal	Enameled	Dry	Nitrogen	Room temp.
19	Frozen	Normal	Enameled	Dry	Air	Room temp.
20	Frozen	Normal	Enameled	Brine	Vacuum	100° F.
21	Frozen	Normal	Enameled	Brine	Vacuum	0° F.
22	Non-irrad. control	Normal	Enameled	Brine	Vacuum	0° F.

[1] The high pH samples refer only to green beans and broccoli.

TABLE 2

The effect of blanching, puréeing and added chemicals on the pigment content and color of sweet potatoes

Treatment	Pigment content			Color		
	Total carotenoids	Beta-carotene	Xanthophyll	Reflectance Rd	Hue $\tan^{-1}a/b$	Chroma $(a^2+b^2)^{1/2}$
	μg./g.	μg./g.	μg./g.			
1. Sliced, unblanched, plain tin cans	359[1]	312	1.70	19.6	35.5	33.4
2. Sliced, blanched, plain tin cans	350	304	1.74	19.3	29.7	31.2
3. Sliced, unblanched, plain tin, KCN dip	469	421	2.67	19.1	41.6	36.8
4. Puréed, unblanched, plain tin	411	359	2.90	22.9	39.6	37.8
5. Puréed, blanched, plain tin	341	296	2.03	21.6	32.3	33.0
6. Puréed, unblanched, plain tin, KCN added	412	358	1.92	22.7	42.4	40.8
7. Puréed, blanched, glass vials	157	125	1.74	14.0	14.6	19.3
8. Puréed, unblanched, glass vials	311	273	2.27	14.8	26.1	22.6
9. Puréed, unblanched, glass vials, chlorogenic acid	309	273	2.59	10.7	18.7	19.0
10. Non-irradiated, sliced, unblanched, plain tin	437	384	2.90	15.9	30.4	28.4
11. Non-irradiated, sliced, blanched, plain tin	383	338	2.40	15.4	21.0	27.1

[1] Each datum was obtained from an average of 8 cans, i.e., 2 at each of 4 storage periods.

total carotenoids, and β-carotene content was determined with an Evelyn colorimeter calibrated for β-carotene whereas for α-carotene the instrument was calibrated with α-carotene purified from a mixture of 85% alpha- and 15% β-carotene.

All pigment contents were calculated as micrograms per gram of dry weight after adjustment for the brine or syrup added. The moisture determinations were performed by drying for 8 hours in a vacuum oven at 70° F.

The color measurements were made with a Gardner Color Difference Meter on samples which had been ground in a meat grinder. A circular 2½ inch diameter opening was used with large area illumination. Pyrex cells with optical glass bottoms were used for the ground samples. Small Plexiglass cells and small area illumination were used for the sweet potatoes in glass vials. The "Rd" value was used as an index of lightness or darkness; $\tan^{-1}a/b$ *(2)* was used for hue and $(a^2+b^2)^{1/2}$ for chroma.

All 4 vegetables were irradiated with 1.8 megarads and stored for approximately ½, 1, 3, 6 and 12 months. The conditions of packing, shipping, irradiation, and time and temperature of storage, have been described by Wishnetsky *(9)*. The conclusions are based on analyses of variance for six groupings at the 1% level of significance.[b]

RESULTS AND DISCUSSION

Overall effects of irradiation and storage. The carotene and xanthophyll content of green beans after irradiation was very variable depending on the conditions. The per cent destruction for carotene ranged from 5% for Treatment No. 10 in Table 1 to 95% for Treatment 19. The irradiated samples stored at 0° F. were about 25% lower in carotene and 22% lower in xanthophyll than the controls stored at the same temperature. The xanthophyll content tended to follow the carotene content as Treatment 10 showed about 20% destruction and Treatment 19, about 95%.

The effect of storage time on the carotene and xanthophyll content of green beans is shown in Figure 1. There was a large initial decrease upon irradiation followed by a rise and then a decrease at the final storage period. At 100° F., xanthophyll was apparently more susceptible to breakdown on storage than carotene because the xanthophyll/carotene ratio decreased from 1.6–2.0 at the beginning of storage to 0.6–0.8 after 48 weeks of storage.

The carotenoid content of broccoli was much less variable than that of green beans upon irradiation. The carotene destruction ranged from 25% for Treatment 18 to 50% for Treatment 3. Xanthophyll destruction ranged from 30% with Treatments 11 and 40 to 60% with Treatment 10. The samples, irradiated frozen and stored frozen, were about 30% lower in both carotene and xanthophyll than the frozen controls.

[b] In the interests of brevity, tables of statistical data were not reproduced here. They will be supplied by the authors upon request.

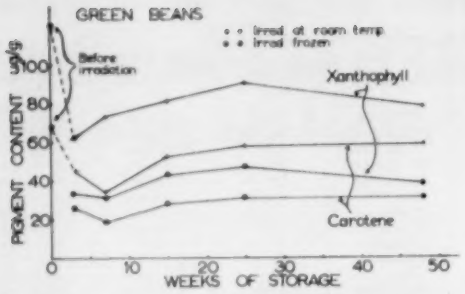


Figure 1. The carotene and xanthophyll content of green beans irradiated at 1.86 megarads and stored under the conditions described in Table 1. Each point represents the average value obtained from duplicate analyses on each of 20 cans.

The effect of storage time on the carotene and xanthophyll content of broccoli is shown in Figure 2. With xanthophyll, there was a large initial drop upon irradiation followed by a rise and a slow decrease upon storage. The carotene content showed little change after the initial drop.

The carotenoid pigments of sweet potatoes showed relatively little destruction by gamma irradiation. The Treatment (No. 7) which resulted in the lowest total carotenoid content was only 20% lower than the non-irradiated controls. The treatment with the greatest carotenoid retention (No. 6) was only 3% lower than the controls. The sweet potatoes irradiated frozen and stored at 0° F. were also about 3% lower than the non-irradiated controls stored at 0° F. The figures for β-carotene content generally ran parallel to the values for total carotenoid content but the figures for xanthophyll were slightly different. Treatment 21, which had the highest β-carotene and total carotenoid content had one of the lowest xanthophyll contents. It might appear that there was an inverse relationship between the xanthophyll and total carotenoid content but the correlation coefficient between the two for all the irradiated samples was not significant.

The effects of storage on total carotenoid and β-carotene content are presented in Figure 3. The storage effects were not very marked and were different for each treatment. In general, the figures for total carotenoid and β-carotene showed an initial decrease upon irradiation followed by a rise and then a slow decline. The xanthophyll content increased with storage.

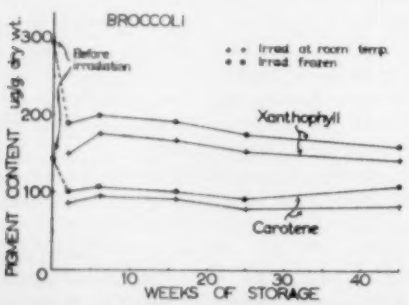


Figure 2. The carotene and xanthophyll content of broccoli irradiated at 1.86 megarads and stored under the conditions described in Table 1. Each point represents the average value obtained from duplicate analyses on each of 20 cans.

The color changes upon storage after irradiation were more marked than the pigment changes. The samples were generally lighter in color except for the samples packed dry and the ones stored at high temperatures which were considerably darker. The hue changed towards the yellow except for the samples in plain tin and the irradiated samples stored frozen where the shift was towards the red. The same samples that were redder also showed an increase in chroma where the other samples showed a decrease when compared with the controls. Upon storage, there was a gradual shift in hue towards the yellow (Figure 3). The color of the sweet potatoes did not correlate with the pigment content, as for example, Treatment 11, which had a redder hue had a relatively low pigment content. The color was influenced by enzymatic discoloration as well as pigment content.

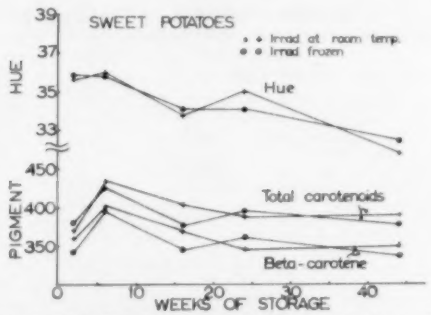


Figure 3. The hue, total carotenoids and beta-carotene in sweet potatoes irradiated at 1.86 magarads and stored under the conditions described in Table 1. Each point represents the average value obtained from duplicate analyses on each of 16 cans. The pigment content is represented as micrograms per gram dry weight and the hue as $\tan^{-1}a/b$.

One incidental observation was that it was much easier to extract the pigments from the irradiated samples than from the controls. This may be because the radiation had degraded the pectin or other constituents of the chloroplasts and plant cells, thus making the pigments more accessible to the solvents.

The carotenoid pigments in carrots showed considerable variation in their response to irradiation depending on the packing conditions. The destruction of total carotenoids ranged from zero for Treatments 11 and 20, to 56% for Treatment 19. The samples irradiated frozen and stored frozen were about 3% lower in total carotenoids than the control samples. In general, the conditions that resulted in the highest total carotenoid retention gave high values for the other pigments as well but did not necessarily result in the greatest retention for each of beta-carotene, alpha-carotene or xanthophyll. Upon storage, the values for total carotenoids, β-carotene, α-carotene and xanthophyll showed a rise at the second and third analysis periods followed by a small decrease (Figure 4).

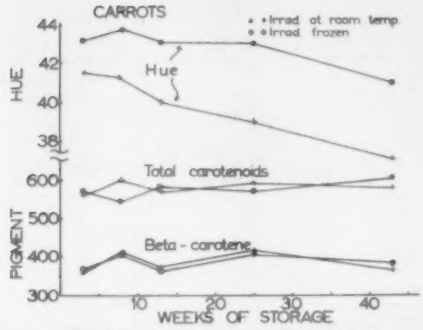


Figure 4. The hue, total carotenoids and beta-carotene in carrots irradiated at 1.86 megarads and stored under the conditions described in Table 1. Each point represents the average value obtained from duplicate analyses on each of 16 cans. The pigment content is represented as micrograms per gram dry weight and the hue as $\tan^{-1}a/b$.

The color of the irradiated samples was darker, yellower, and less saturated, depending on the conditions of storage. The non-irradiated controls had the highest Rd readings followed by the samples irradiated frozen and stored frozen then the samples in plain tin. The darkest samples were those stored at 100° F. Apparently, there was a darkening process occurring which was more evident as the storage temperature increased. Similarly, the samples irradiated at room temperature and stored at 100° F. showed the greatest shift in hue towards the yellow. The irradiated samples stored frozen and the plain tin samples irradiated frozen had a more orange hue than the controls but all other samples were yellower. The same general trends were evident with the chroma values. The lowest values were obtained with the samples stored at high temperatures. The samples irradiated and stored frozen had chroma values similar to the controls and the other treatments were intermediate between the two. The overall color values did not correlate with the pigment content because of the darkening effect on storage.

The treatment which showed the greatest carotenoid breakdown for all 4 vegetables was a dry pack in air. It is well known that the carotenoid pigments are susceptible to oxidation and apparently the irradiation process facilitated the oxidation. This would agree with the hypothesis of Lukton and Mackinney *(5)* that the destructive effects of irradiation on carotenoid pigments are mainly indirect.

One consistent trend was the increase in carotenoid content at the second or third period of analysis. The trend was significant for total carotenoids, beta-carotene, alpha-carotene and xanthophyll in carrots; total carotenoids, beta-carotene and xanthophyll in sweet potatoes; and carotene and xanthophyll for both green beans and broccoli. The increase was not evident in the experiments on enzyme action in sweet potatoes which were packed in early spring after being in bulk storage for some months. It is well known that sweet potatoes will synthesize carotenoids on storage *(1)* but this could hardly explain the increase in blanched green beans and broccoli.

Effects of temperature of product during irradiation. With green beans, irradiation in the frozen state resulted in greater destruction of both carotene and xanthophyll (Figure 1). With broccoli, the situation was reversed as irradiation in the frozen state gave greater retention of both carotene and xanthophyll (Figure 2). With sweet potatoes, the irradiation temperature did not affect the pigment content or the hue but irradiation at room temperature produced samples with higher reflectance and chroma values (Figure 3). With carrots, the irradiation temperature did not affect the total carotenoids, β-carotene or α-carotene content (Figure 4). However, irradiation at room temperature resulted in a product with a lower content of xanthophyll, a lower reflectance, a yellower hue and a lower chroma.

The effect of temperature of irradiation was seldom a clear cut main effect but was nearly always complicated by significant interactions between one or more of the other packing conditions.

Effects of headspace gas. Green beans and broccoli packed dry in air showed severe carotenoid destruction (Table 3). The vacuum packs showed fairly good retention and the nitrogen packs were somewhat lower. With sweet potatoes packed dry, the nitrogen packs showed the highest pigment retention followed by the vacuum samples and last by the air packs. The color of the sweet potatoes was also significantly affected by the headspace gas. The vacuum packs had the highest reflectance values followed by the samples in nitrogen and then in air. The samples in air had a yellower hue than the other two. The vacuum packed samples had the highest chroma followed by the samples in nitrogen and then in air. With carrots, packed dry, the vacuum packs showed the highest

TABLE 3

The carotenoid content of four vegetables packed dry in enameled cans and stored at room temperature for eleven months

	Irradiated at room temp.			Irradiated frozen		
	Vacuum	Nitrogen	Air	Vacuum	Nitrogen	Air
Green Beans[1]						
Carotene[2].....	52[4]	30	7	39	11	3
Xanthophyll[2]...	84	44	9	60	13	7
Broccoli[1]						
Carotene.....	95	81	78	103	111	92
Xanthophyll....	155	157	129	174	213	179
Sweet Potatoes						
Total carotenoids.....	359	393	374	396	417	371
β-carotene.....	325	354	339	363	382	338
Xanthophyll....	1.72	1.75	1.65	2.04	2.03	1.67
Reflectance[3]....	19.7	18.9	18.2	20.4	18.1	17.0
Hue[3].....	34.8	34.3	29.9	34.0	33.0	29.6
Chroma[3].....	27.2	26.8	24.8	27.0	25.2	23.0
Carrots						
Total carotenoids.....	574	496	344	585	415	318
β-carotene.....	352	326	222	377	259	208
α-carotene.....	109	84	58	115	75	53
Xanthophyll....	6.33	5.35	6.43	5.38	4.61	3.93
Reflectance.....	12.0	12.5	12.9	12.9	14.5	15.3
Hue.....	41.3	40.8	37.6	40.9	42.2	39.5
Chroma.....	30.4	30.2	28.7	31.4	32.5	30.4

[1] Color and chlorophyll values for these cans were published in a previous paper *(9)*.

[2] Pigment contents in μg./g. dry weight.

[3] The reflectance, hue and chroma were represented by Rd, $\tan^{-1}a/b$, and $(a^2 + b^2)^{1/2}$ respectively.

[4] Each datum was obtained from an average of 10 cans, i.e., 2 at each of five storage periods.

pigment retention followed by the nitrogen packs and then the samples in air. The headspace gas also affected the color of carrots as the vacuum and nitrogen packs had higher hue and chroma values and lower Rd values than the air packs.

When the vegetables were packed in brine or water, the headspace gas was less important (Tables 4 and 5). Carotenoid retention and color in all 4 vegetables was similar for both nitrogen and vacuum headspaces in nearly every case.

Effects of packing media. Green beans packed in water showed higher carotenoid retention than the brine packs when irradiated in the frozen state but not when irradiated at room temperature (Table 4). With broccoli, both water and brine packs were similar in carotenoid content. With sweet potatoes, the syrup packs showed higher pigment retention than the water packs. The hues and chromas of the 2 packs were similar but the water packs were darker. With carrots, the pigment content of the brine packs was slightly higher than the water packs but the colors were similar.

Effects of can lining. In green beans, there was no significant difference in the carotenoid content due to can lining when the samples were irradiated at room temperature (Tables 4 and 5). With irradiation in the frozen state, plain tin cans showed greater carotenoid retention, particularly in the latter part of the storage period. With broccoli, plain tin cans gave better carotenoid retention. With sweet potatoes and carrots, there was no difference in pigment content due to can lining, but the samples in plain tin were lighter, more orange in color and had a higher chroma than the samples in enameled cans. Since the can lining had a highly significant effect on the color without influencing the pigment content, it would appear that the two are not necessarily related.

Effects of storage temperature. The samples of green beans stored at 100° F. showed surprising carotenoid retention as the samples irradiated at room temperature had a carotene content even higher than the samples irradiated frozen and stored frozen (Table 6). On the other hand, the samples irradiated

TABLE 4

The carotenoid content and color of four vegetables packed under vacuum in enameled cans and stored at room temperature for eleven months

	Irradiated at room temp.		Irradiated frozen	
	Brine	Water	Brine	Water
Green Beans[1]				
Carotene[2]	63[4]	61	16	52
Xanthophyll[2]	101	97	17	57
Broccoli[1]				
Carotene	75	85	99	106
Xanthophyll	131	151	187	186
Carrots				
Total carotenoids	705	657	653	642
β-carotene	440	413	422	407
α-carotene	133	128	123	128
Xanthophyll	6.6	6.5	7.9	7.1
Reflectance[3]	10.8	10.8	13.9	14.0
Hue[3]	39.0	39.6	43.4	43.3
Chroma[3]	28.4	28.6	34.5	33.8
Sweet Potatoes	Syrup	Water	Syrup	Water
Total carotenoids	403	422	377	416
β-carotene	366	382	339	383
Xanthophyll	1.74	1.81	1.40	1.77
Reflectance	19.7	21.7	18.9	20.5
Hue	38.9	37.8	39.9	39.7
Chroma	30.4	29.1	29.9	29.1

[1] Color and chlorophyll values for these cans were published in a previous paper (*9*).
[2] Pigment contents in μg./g. dry weight.
[3] The reflectance, hue and chroma were represented by Rd, $\tan^{-1}a/b$, and $(a^2 + b^2)^{1/2}$ respectively.
[4] Each datum was obtained from an average of 10 cans, i.e., 2 at each of five storage periods.

TABLE 5

The color and pigment content of four vegetables packed in brine and stored for eleven months at room temperature

	Plain tin cans		Enameled cans	
	Vacuum headspace		Nitrogen headspace	
	Irrad. at R.T.	Irrad. frozen	Irrad. at R.T.	Irrad. frozen
Green Beans[1]				
Carotene[2]	61[4]	40	48	22
Xanthophyll[2]	97	65	71	26
Broccoli[1]				
Carotene	94	110	110	100
Xanthophyll	176	215	210	189
Carrots				
Total carotenoids	640	717	647	665
β-carotene	400	457	406	405
α-carotene	133	138	127	131
Xanthophyll	5.01	6.66	6.98	7.62
Reflectance[3]	13.9	16.7	11.3	13.9
Hue[3]	43.5	45.7	40.5	44.1
Chroma[3]	33.8	38.1	29.4	33.7
Sweet Potatoes (syrup pack)				
Total carotenoids	402	381	409	367
β-carotene	368	348	370	337
Xanthophyll	1.88	1.66	1.62	1.67
Reflectance	21.6	21.0	19.1	18.4
Hue	41.0	41.6	39.3	38.5
Chroma	32.4	32.5	30.6	29.0

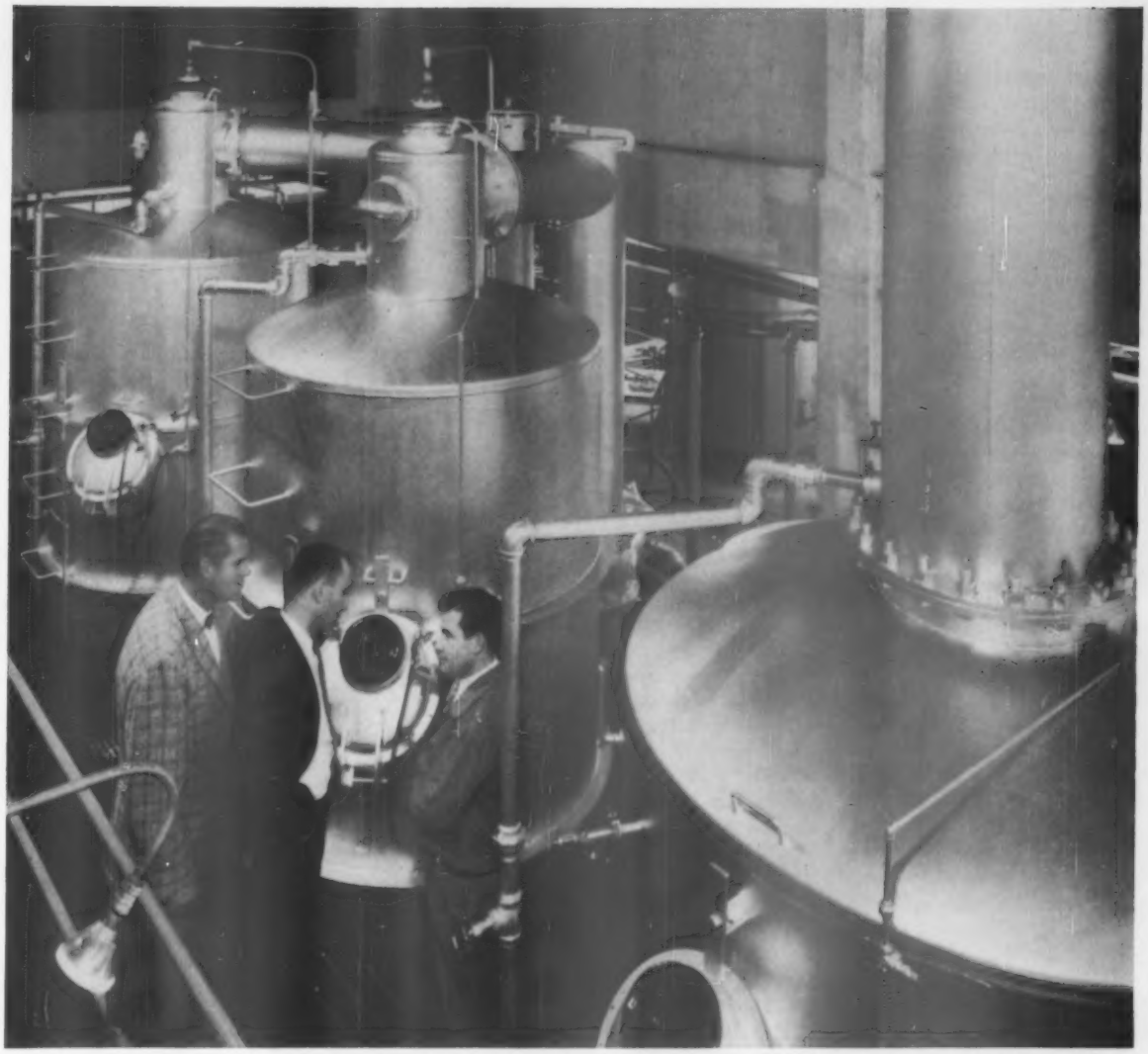
[1] Color and chlorophyll values for these cans were published in a previous paper (*9*).
[2] Pigment contents in μg./g. dry weight.
[3] The reflectance, hue and chroma were represented by Rd, $\tan^{-1}a/b$, and $(a^2 + b^2)^{1/2}$ respectively.
[4] Each datum was obtained from an average of 10 cans, i.e., 2 at each of five storage periods.

TABLE 6

The effect of storage temperature on the carotenoid content and color of four vegetables packed in brine under vacuum in enameled cans

	Storage temperature				
	0° F.	70° F.		100° F.	
	Irrad. frozen	Irrad. at R.T.	Irrad. frozen	Irrad. at R.T.	Irrad. frozen
Green Beans[1]					
Carotene[2]	59[4]	63	16	73	23
Xanthophyll	102	101	17	101	30
Broccoli[1]					
Carotene	104	75	99	86	95
Xanthophyll	221	131	187	129	146
Carrots					
Total carotenoids	692	705	653	693	719
β-carotene	421	440	422	436	429
α-carotene	144	133	123	140	136
Xanthophyll	6.9	5.0	7.9	6.7	8.1
Reflectance[3]	17.3	10.8	13.9	9.8	10.2
Hue[3]	47.1	39.0	43.4	39.0	42.1
Chroma[3]	39.5	28.4	34.5	28.4	29.4
Sweet Potatoes (syrup pack)					
Total carotenoids	424	403	377	419	400
β-carotene	386	366	339	380	354
Xanthophyll	1.4	1.7	1.4	1.8	1.8
Reflectance	21.6	19.7	18.9	15.6	16.3
Hue	44.1	38.9	39.9	34.8	34.6
Chroma	34.5	30.4	29.0	27.4	27.6

[1] Color and chlorophyll values for these cans were published in a previous paper (*9*).
[2] Pigment contents in μg./g. dry weight.
[3] The reflectance, hue and chroma were represented by Rd, $\tan^{-1}a/b$, and $(a^2 + b^2)^{1/2}$ respectively.
[4] Each datum was obtained from an average of 10 cans, i.e., 2 at each of five storage periods.



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in the frozen state and stored at 100° F. had a very low carotene content. With broccoli, high temperature storage resulted in greater destruction of xanthophyll but there was little effect on carotene. With sweet potatoes and carrots, the samples stored at 0, 70, and 100° F. had similar pigment contents.

The temperature of storage had a marked effect on the color of sweet potatoes and carrots. The samples at the higher temperature were much darker, lower in chroma and showed the greatest hue shift towards the yellow (Table 6).

Effects of pH. Green bean samples packed with a high pH brine showed some carotenoid destruction particularly with the xanthophyll content when packed in enamel cans and irradiated at room temperature. In general, however, adjusting the pH of the brine was not an important variable for the carotenoid content of green beans or broccoli. The effect of adjusting the pH value was not investigated for sweet potatoes or carrots.

Effects of blanching and puréeing. A separate experiment was set up to investigate the effect of blanching, puréeing and chemical enzyme inhibitors since Nishida and coworkers *(6)* reported that sweet potatoes contain an enzyme system that destroys carotene. The data are reported in Table 2.

The blanching process was particularly destructive with carotenoids as all blanched samples were lower in pigment content. If the loss in pigment content was due to enzyme action, blanching would be expected to minimize the loss but this apparently did not happen. The puréeing process also reduced the pigment content probably because of the added air incorporated into the product even though the samples were packed under vacuum. The lowest pigment content in this series was found in the blanched, puréed samples in glass vials (36% of pigment content of unblanched non-irradiated controls). Apparently, the blanching and puréeing effects were additive.

The blanching process also changed the color of the sweet potatoes. The hues, particularly of the blanched samples, were yellower but the chroma and reflectance values were also lower. The puréeing process also changed the color in the same manner, and the overall color changes were most marked in the samples which were both puréed and blanched (Treatment 7 in Table 2).

Effects of type of container and storage time. The container had a significant effect on the pigment content and color of the sweet potatoes (Table 2). The samples in plain tin cans had a much higher pigment content than the samples packed in glass vials and showed very little pigment loss on storage. The samples in glass vials showed more initial destruction and even more loss on storage. The samples in cans had higher Rd, hue and chroma values than the samples in vials. Generally, the Rd value increased with storage indicating that the samples became lighter in color, whereas the hue and chroma decreased, indicating a shift towards the yellow and a less intense color.

Effects of chlorogenic acid and potassium cyanide. Chlorogenic acid has been reported to inhibit the oxidation of β-carotene *(7)* but it did not protect the pigment from destruction in this experiment. It would appear that the loss in carotene through enzymic action is relatively small. The chlorogenic acid also changed the color appreciably as this set of samples had the lowest values for reflectance, hue and chroma and were the least attractive in appearance.

Potassium cyanide was added to some cans as a general enzyme inhibitor but it apparently had other effects as well because the pigment content and color was better than that in the non-irradiated frozen controls. The puréed samples with potassium cyanide added and also the sample where the slices were dipped in a solution of potassium cyanide were very high in pigment content. In addition, they had surprisingly high values for hue and chroma. These samples were much more orange than the frozen controls and were very attractive in appearance. The mechanism of this reaction merits further study.

Optimum conditions for pigment retention and color. In Table 7, are listed the 10 treatments with the highest carotenoid retention in irradiated green beans and broccoli. A similar table for chlorophyll content and color has been published in a previous paper *(9)*. In Table 8, are listed the 10 best treatments for sweet potatoes and carrots from a color and pigment point of view. It was assumed that a higher value for hue and chroma represented a more attractive color. With the reflectance values for sweet potatoes the choice is not so clear cut because a high value may indicate a slightly bleached product. However, it was assumed, in the absence of published data on the optimum color for consumer acceptance, that the lighter colored samples would be more acceptable than the darker ones.

It would appear from Table 8, that the optimum conditions for pigment retention in sweet potatoes were not the same as for color. Treatment No. 1 where the sweet potatoes were packed in syrup under vacuum in plain tin cans and irradiated at room temperature would be a good compromise if hue were

TABLE 7

Ten treatments for green beans and broccoli with the highest carotenoid retention

Green beans		Broccoli	
Carotene μg./g. dry wt.	Xanthophyll μg./g.	Carotene μg./g.	Xanthophyll μg./g.
73[1] (10)[2]	101 (10)	111 (18)	215 (11)
63 (2)	101 (2)	110 (11)	215 (14)
63 (3)	101 (3)	110 (5)	213 (18)
61 (1)	97 (1)	108 (14)	210 (5)
61 (6)	97 (6)	106 (16)	196 (12)
52 (16)	84 (7)	103 (17)	189 (15)
52 (7)	77 (16)	102 (12)	187 (13)
48 (5)	71 (5)	100 (15)	186 (16)
46 (12)	65 (11)	99 (13)	179 (19)
44 (4)	63 (12)	98 (2)	176 (1)
59 (21)[3]	102 (21)	104 (21)	221 (21)
77 (22)[3]	125 (22)	151 (22)	312 (22)

[1] Each datum represents the average obtained from 10 cans, i.e., duplicate analyses at each of five storage periods.

[2] The figures in brackets represent the treatment numbers as described in Table 1.

[3] Treatments 21 and 22 represent the irradiated and non-irradiated samples stored at zero °F.

TABLE 8

Ten treatments for irradiated sweet potatoes and carrots with the least change in color and pigment content

Color			Pigment content			
Hue $\tan^{-1}a/b$	Reflectance Rd	Chroma $(a^2+b^2)^{1/2}$	Total carotenoids	Beta carotene	Alpha carotene	Xanthophyll
			μg./g. dry wt.	μg./g.	μg./g.	μg./g.
			Sweet Potatoes			
41.6[1] (11)[2]	21.7 (6)	32.5 (11)	422 (6)	383 (18)		2.04 (17)
41.0 (1)	21.6 (1)	32.4 (1)	419 (10)	383 (16)		2.03 (18)
39.9 (13)	21.0 (11)	30.4 (3)	417 (18)	382 (6)		1.88 (1)
39.7 (16)	20.5 (16)	30.3 (5)	416 (16)	380 (10)		1.81 (6)
39.3 (5)	20.4 (17)	29.9 (13)	409 (5)	370 (5)		1.79 (20)
38.9 (3)	19.7 (7)	29.1 (16)	403 (3)	368 (1)		1.77 (16)
38.5 (15)	19.7 (3)	29.1 (6)	402 (1)	366 (3)		1.76 (10)
37.8 (6)	18.9 (8)	29.0 (15)	400 (20)	363 (17)		1.75 (8)
34.8 (7)	18.9 (13)	27.6 (20)	396 (17)	354 (20)		1.74 (3)
34.3 (8)	18.4 (15)	27.4 (10)	393 (8)	354 (8)		1.72 (7)
44.1 (21)[3]	21.6 (21)	34.5 (21)	424 (21)	386 (21)		1.43 (21)
39.8 (22)[3]	18.6 (22)	29.5 (22)	435 (22)	404 (22)		1.27 (22)
			Carrots			
45.7 (11)	16.7 (11)	38.1 (11)	719 (20)	457 (11)	140 (10)	8.09 (12)
44.1 (15)	15.3 (19)	34.5 (13)	717 (11)	440 (3)	138 (11)	7.92 (13)
43.5 (1)	14.5 (18)	33.8 (1)	705 (3)	436 (10)	136 (20)	7.62 (15)
43.4 (13)	14.0 (16)	33.8 (16)	693 (10)	429 (20)	133 (3)	7.11 (16)
43.3 (16)	13.9 (1)	33.7 (15)	665 (15)	422 (13)	131 (15)	6.98 (5)
42.2 (18)	13.9 (13)	32.5 (18)	657 (6)	413 (6)	128 (6)	6.66 (10)
42.1 (20)	13.9 (15)	31.4 (10)	653 (13)	407 (16)	128 (16)	6.66 (11)
41.3 (7)	12.9 (17)	30.4 (19)	647 (5)	406 (5)	127 (5)	6.62 (3)
40.9 (17)	12.9 (17)	30.4 (7)	642 (16)	405 (15)	123 (13)	6.49 (6)
40.8 (8)	12.5 (8)	30.2 (8)	640 (1)	400 (1)	120 (1)	6.43 (9)
47.1 (21)	17.3 (21)	39.5 (21)	692 (21)	441 (21)	144 (21)	6.75 (21)
44.4 (22)	19.3 (22)	40.6 (22)	719 (22)	464 (22)	149 (22)	6.04 (22)

[1] Each datum represents the average of 10 cans, i.e., duplicate analyses for each of five storage periods.
[2] The figures in brackets represents the treatment numbers as described in Table 1.
[3] Treatments 21 and 22 represent the irradiated and non-irradiated samples stored at zero °F.

more important than pigment content. If it were felt that the reverse was true, Treatment 16 might be the logical choice.

With irradiated carrots, the optimum conditions for pigment retention were also the optimum conditions for color. Treatment No. 11 where the carrots were packed under vacuum in brine in plain tin cans and irradiated in the frozen state, would be the logical choice.

Both carrots and sweet potatoes when irradiated under optimum conditions produced products that would compare very favorably from a color and pigment retention point of view with conventional heat treated or frozen products.

SUMMARY

The color and pigment retention in irradiated sweet potatoes and carrots and the carotenoid retention in irradiated green beans and broccoli have been investigated over one year's storage time with a variety of packing conditions. The chief findings were as follows:

1. The destruction of carotene and xanthophyll in green beans upon irradiation ranged from 5 to 95% depending on the conditions. The greatest loss was found in beans packed dry in air and irradiated frozen. The least destruction was found in samples packed in brine under vacuum irradiated at room temperature and stored at 100° F.

2. The destruction of carotene and xanthophyll in broccoli ranged from 25 to 50%. The greatest retention was found with samples packed dry under nitrogen and irradiated in the frozen state.

3. The destruction of carotenoids in sweet potatoes ranged from 3 to 20%. The samples with the greatest retention were packed in water under vacuum and irradiated at room temperature.

4. The destruction of carotenoids in carrots ranged from zero to 56%. The samples with the greatest retention were packed in brine under vacuum in plain tin cans and irradiated in the frozen state. These conditions also resulted in the most attractive color for both carrots and sweet potatoes.

5. Upon storage, there was usually an initial decrease in pigment content followed by a rise and then a slow decline. The hues of sweet potatoes and carrots gradually changed towards the yellow upon storage and the samples became darker.

6. Temperatures of irradiation, headspace gas, packing media, can lining and temperature of storage all had a small effect on the color and carotenoid content of the four vegetables.

7. Blanching or puréeing resulted in a much lower pigment content in irradiated sweet potatoes.

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Factors Affecting the Water Retention of Beef

I. Variations in Composition and Properties among Eight Muscles*

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Considerable interest in identifying the factors which affect the water retention of meat has recently been in evidence in the meats field. The practical goal of this research is the development of means of controlling and, when desired, enhancing the capacity of meat to retain water or juice. Present knowledge permits this to only a limited extent.

Results of previous studies indicate that pH markedly affects water retention and, also that electrolytes exert an influence. The pronounced effect of pH value has been reported as a result of studies dealing with meat only *(8, 9, 15, 18)*, and with meat containing additives *(12, 22, 30)*. Known causes for variation in the pH values of meat include differences in lactic acid formation after slaughter *(3, 19)*, in beef from animals of different age *(20)*, and during the aging of meat *(19, 31)*. Muscles of both the pig *(27)* and the horse *(19)* have been found to have different ultimate pH values, "ultimate" pH being the low pH attained in the post rigor muscle. It has been reported that the effect of calcium and zinc on the hydration of meat proteins is to decrease their water retention *(11)*. The effect of the ions appears to be appreciably influenced by pH, which has been reported to determine the amount and direction of ion effects *(11)*, and by the presence of ion-complexing agents such as the polyphosphates *(10)*. It should be noted that muscle and meat pH values in this paper and in the literature cited are the pH values of extracts or juice given opportunity to equilibrate to some degree with muscles or meat proper. A basis for assuming that the movement of ions during post mortem aging is related to the hydration and tenderness of meat has been reported *(1)*. The information available falls short of explaining differences in meat as reflected by variations in water retention, juiciness, and tenderness which, in part, is also thought to be affected by water retention. New light shed on variations in meat as regards both composition and properties is needed to improve this situation.

Previous investigations of water retention were made by different methods and generally have involved determination of the effects of variations in composition indirectly by the addition of salt solutions, adjustment of pH with acid, alkali, or buffers, and, on occasion, adjustment of ion content with complexing agents. In the present study a different approach was used, involving a comparative study

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of the properties of muscles from the same and different animals. By this means, variables related to the history of the samples were eliminated and variation in composition and properties was obtained, without introducing extraneous materials, with the exception of water. The experiments involved determining water retention, pH values, electrolyte composition (calcium, magnesium, zinc, iron, phosphorus, chloride, sodium, and potassium), and the proximate composition of eight muscles from each of four animals and pH values and water retention of eight muscles from an additional animal. These results provide new information on variations to be expected in the composition and properties of muscles from the same, as well as from different, animals.

The results were also used in investigating the pattern of the pH values and water retention of the series of muscles and in investigating relations between water retention and composition. The inability of the correlations attempted to indicate the independent effects of variation of components was recognized, as was the fact that interpretation of trends based on analyses of total contents has obvious limitations that will be dealt with later in detailed studies. However, by considering both the degree and direction of the correlations of the relationships investigated, the results provided new information on the relations between meat composition and water retention and indicated promising areas in need of further investigation.

EXPERIMENTAL AND RESULTS

Animals and muscles used. Carcasses of two cows, a bull, a heifer, and a steer were obtained immediately after slaughter, and the 8 muscles named in Table 1 were separated. Separation of the muscles from the carcasses and removal of separable fat and connective tissue required from 1 to 2 hours, measured from the time of slaughter. Each muscle was cut into cubes approximately 16 cc. in size, and the cubes were thoroughly mixed.

TABLE 1

Muscles selected and locations

Muscle		Location	
Number		Fore quarter	Hind quarter
1	*Longissimus dorsi*	Rib, chuck	Sirloin, porterhouse
2	*Psoas*		Sirloin, porterhouse
3	*Semimembranosus*		Round
4	*Serratus ventralis* (thoracic part)	Rib, chuck	
5	*Rectus abdominus*	Plate	Flank
6	*Semitendinosus*		Round
7	*Latissimus dorsi*	Rib, chuck	
8	*Trapezius*	Rib, chuck	

Determination of water retention. The procedure applied to samples of each muscle was as follows: A suitable number of cubes was ground through a grinder equipped with a plate having approximately 5 mm. openings. Each of two 15 g. portions of sample was weighed into tared cellulose centrifuge tubes (40 ml. capacity) and 15 ml. of cold, distilled water (0° C.) were added to each tube. Tissue and water were well mixed with a stirring rod and the mixtures were stored overnight at 0° C. Caps were placed on the tubes, approximately 8 ml. of distilled water were added to each tube, and the mixtures were centrifuged in a Spinco Model L,[b] fitted with head No. 30, for 20 minutes at 15,000 r.p.m. The supernatant liquid was decanted from the tubes which were inverted to drain for 5 minutes. The pH values of the supernatant fractions were determined using a Beckman Model GS pH meter. The drained tubes and meat were weighed and the differences between the original and final weights were calculated and averaged. In the calculations loss of extractives in the supernatant liquid were disregarded. The mean change in weight of the samples is reported as water retention, △ wt./15 g. meat.

Analytical methods. Modifications of the basic analytical methods used will be described in a separate report *(6)*. The following describes the general methods employed in analyses of muscles from all the animals, except those of the heifer, which were only used in the investigation of the relation of pH and water retention.

A portion of the cubed sample obtained from each muscle was finely comminuted and mixed in a Servall Omni-mixer. The comminuted sample resembled a fine paste. Fifteen-gram aliquots of the paste were weighed into quartz crucibles and dried to constant weight by heating them for approximately 16 hours at 105° C. The samples were then charred by heating at 350° C. and ashed overnight at 550° C. *(21)*. The ash was dissolved in 6 N HCl and diluted to volume. Aliquots of the stock solution were used in determining sodium, potassium, calcium, magnesium, zinc, iron, and phosphorus as follows: Sodium and potassium were determined by flame photometry *(4)*, zinc as the dithizonate in carbon tetrachloride solution *(26)*, iron as the ortho-phenanthrolene complex *(2)*, phosphorus as molybdivanadophosphoric acid *(17)*, calcium as the oxalate *(5, 29)*, and magnesium with EDTA *(16, 23)* subtracting the contribution of calcium. Fat was determined by a modified Babcock procedure on separate 9-g. samples of meat paste *(25)*. Chloride was determined on separate 10-g. samples of meat paste using a modified Volhard method *(7, 32)*. Moisture content was determined by the oven drying method *(33)*.

Average values obtained from pooled data on the fat, moisture, and protein (N% x 6.25) content of the muscles and the results of calculations of moisture-to-protein ratios based on these data are shown in Table 2. Averages of data obtained in determinations of electrolytes are summarized in Tables 3 and 4. The data obtained on determining water retention and the pH values of water extracts of muscles from 5 animals are shown in Table 5, along with the results of a statistical analysis of differences between individual muscles. The results of calculations of the correlation coefficients of relationships between components and water-holding capacity are given in Table 6.

TABLE 2

Average moisture, fat, and protein content and moisture/protein ratios of 8 muscles from 4 animals

Muscle	Moisture content	Fat content	Protein (N x 6.25) content	Moisture: protein ratio
	%	%	%	
1. *Longissimus dorsi*	74.15	2.48	22.60	3.28
2. *Psoas*	72.95	3.83	20.60	3.54
3. *Semimembranosus*	73.90	1.85	22.10	3.34
4. *Serratus ventralis*	70.90	8.30	17.90	3.96
5. *Rectus abdominus*	73.20	4.80	21.13	3.47
6. *Semitendinosus*	74.20	2.10	21.95	3.38
7. *Latissimus dorsi*	74.75	2.53	21.18	3.53
8. *Trapezius*	73.90	2.45	20.50	3.61

DISCUSSION

Muscles used in the investigation are named and their location in ordinary meat cuts is given in Table 1. At the outset of the work the only indication of

[b] The mention of specific trade names throughout this paper does not constitute endorsement of the product used over comparable equipment.

TABLE 3

Average content of electrolytes of 8 beef muscles from 4 bovine animals, mg./100 g.

Muscle	Ca	Mg	Zn	Na	K	Fe	P	Cl
Longissimus dorsi	4.30	21.99	3.63	43.0	415	2.73	172	40.3
Psoas	4.03	23.13	2.13	43.8	383	2.60	179	44.2
Semimembranosus	4.14	23.12	3.68	39.5	411	2.85	170	42.9
Serratus ventralis	3.26	17.09	7.37	52.7	336	3.51	154	55.0
Rectus abdominus	4.07	19.99	5.71	53.2	393	2.55	176	55.7
Semitendinosus	4.40	22.47	2.83	42.7	436	2.16	170	48.5
Latissimus dorsi	3.74	21.71	4.66	49.0	405	2.56	184	53.7
Trapezius	4.28	19.32	3.64	64.3	381	2.60	164	72.9

TABLE 4

Content of electrolytes in beef muscles

	Ion content, mg./100 g. muscle							
	Ca	Mg	Zn	Na	K	Fe	P	Cl
	(1, 6)	(1, 2, 3)	(1, 5)	(1, 2)	(1, 2)	(1, 4)	(6)	
Literature, beef, general	2.6–12.0	19.0–31.8	4.7–6.1	41–67	300–338	1.5–3.7	131–186	
Found, range, individual muscles	2.7– 5.6	14.0–25.7	1.2–8.3	36–85	297–451	1.6–5.5	109–213	34–91
Found, average, all muscles	4.0	21.1	4.2	58.5	395	2.7	171	51.6

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TABLE 5

pH values and water retention of muscles

Muscle no.	pH		Water retention	
	Average value	Statistical significance	Average wt./15 g. meat	Statistical significance
4	5.79		−.85	
5	5.68		−1.74	
8	5.60		−2.51	
7	5.59		−2.59	
2	5.53		−2.92	
1	5.53		−2.99	
3	5.51		−3.01	
6	5.50		−3.13	

[1] The values (pH and water retention) for muscles opposite a bar are different from those not opposite the same bar (calculated from data on five animals, statistically significant at the 5% level, 8 degrees of freedom).

TABLE 6

Results of statistical analyses of relationships between properties of 8 muscles

Component	Correlation with water retention		Correlation with protein (N% x 6.25) content	
	r	P[1]	r	P[1]
pH	+0.947 ± .028	.01	−0.892 ± .056	.01
Zn	+0.912 ± .046	.01	−0.631 ± .165	.10
Fe	+0.648 ± .160	.10	−0.709 ± .178	.05
Na	+0.634 ± .165	.10	−0.525 ± .199	n.s.
Moisture	−0.718 ± .133	.05	.....	.....
Protein	−0.854 ± .074	.01	.....	.....
Moisture, %/ protein, %	+0.861 ± .071	.01	.....	.....
Fat	+0.868 ± .067	.01	.....	.....
Cl	+0.519 ± .201	n.s.	−0.456 ± .218	n.s.
Ca	−0.779 ± .108	.05	+0.827 ± .087	.01
Mg	−0.952 ± .038	.01	+0.806 ± .097	.02
K	−0.811 ± .094	.01	+0.937 ± .033	.01
P	−0.544 ± .194	n.s.	+0.604 ± .175	.10

[1] 6 degrees of freedom.

possible differences in the muscles was the various degrees of tenderness attributed to them by Ramsbottom *(24)*. While differences in tenderness need not indicate differences in water-holding capacity and other properties, the limited guidance this provided proved useful as a basis for selection. As the present results indicate, sufficient variation in properties existed to satisfy the needs of the investigation.

The results of the proximate analysis and the calculations of moisture: protein ratios for the different muscles are given in Table 2. As indicated by the data, the muscles were low in fat and high in protein and moisture content, and tended towards leanness with the exception of the *serratus ventralis* muscles, which had some visible, but not readily separable, fat between bundles of muscle fibers. The results of the determination of calcium, magnesium, zinc, sodium, potassium, iron, phosphorus, and chloride are reported in Table 3. As the results indicate, the content of electrolytes of individual muscles ranged widely. A comparison of the average content of electrolytes in the muscles with values reported in the literature for meat is given in Table 4. The values previously reported and those found are substantially in agreement.

The 8 muscles of given animals are arranged in Table 5 in order of decreasing average pH values and water retention. The differences between the average pH values of the *serratus ventralis* (muscle 4) and the *rectus abdominus* (muscle 5), and between the pH values of these two muscles and those of the other six, were statistically significant. The average water retention of the *serratus ventralis* muscles was significantly larger than the average values obtained for the other muscles. Otherwise, differences between pairs of muscle pH values or between the water retention of pairs of muscles were not significant.

Analyses of trends of relations among the properties of the eight muscles were made to provide a statistical basis for interpreting results. The animals were slaughtered over a period of several months. As work on muscles of the individual animals was completed

the data obtained were analyzed. The statistical analyses involved calculating linear regression equations and coefficients, and testing the significance of correlation coefficients. The results of calculations based on the data obtained on individual animals indicated that the same relationships were significant in each case. Subsequently, the data on each of the 8 muscles of the 4 animals (5 animals in the case of the pH values and the values for water retention reported in Table 5) were averaged, and the averaged data were statistically analyzed. The relationships derived from the averaged data completely agreed with those based on data obtained on the muscles of individual animals, except that the correlation coefficients obtained on analysis were somewhat higher. These averaged data would yield significant correlations only if the relative order of the muscles, with regard to water retention and to other properties, tended to follow the same pattern from animal to animal. Following this procedure, a number of significant correlations between properties of the muscles were found; hence, the results indicate that certain differences in the muscles of the animals followed a pattern.

The data in Table 6 show the results of statistical analyses of the relations between the water retention and components of the muscles, and between protein content (N% x 6.25) and the content of other components. The results indicate that water retention was inversely related to protein content (N% x 6.25). Further, as is shown, the correlations could be separated into two groups; one, of components which varied positively with variations in water retention and negatively with variations in protein content, and another, of components which varied negatively with variations of water retention and positively with variations of protein content. The data generally indicate that components positively associated with increasing water retention were negatively associated with increasing protein content and *vice versa*. A second generality of possible significance is that components which may be considered as intracellular components, including calcium, magnesium, potassium, phosphorus, and the bulk of the protein, were found to be inversely associated with water retention, while the trend of sodium and chloride, which are mainly expected to be components of extra-cellular fluids, were found to be directly associated with water retention.

The negative correlation between water-retention and protein content, in which protein content was calculated using the generally based relation, per cent N x 6.25 equals per cent protein, was highly significant ($r = 0.854 \pm .074$, $P < .01$). This inverse relation is paradoxical, inasmuch as protein is the component of muscle assumed to be principally responsible for water retention. Interpretation of the results involves consideration of the validity of the asumption that content of nitrogen and protein in the 8 muscles had a constant relation, whether by a factor of 6.25 or by any other. Inasmuch as minor variation in the proportion of protein to non-protein nitrogenous substances would not affect the inverse relation found between water retention and protein content, and the occurrence of wide variations has not, as yet, been reported, the validity of expressing per cent N x 6.25 as per cent protein, in this case has been tentatively accepted. In rigorous aspect it should, however, be noted that nitrogen, not protein, was determined.

As shown by the data given in Table 6, increasing pH values were found to be closely correlated with increasing water retention ($r = .947 \pm .028$, $P < .01$). This confirms the results previously reported *(8, 9, 15)*. Assuming pH to be the significant parameter and the pH of the series of muscles to be inversely related to protein content provides an explanation for the paradoxical relation of protein content to water retention. Investigations are in progress to obtain information on the relative proportions of different constituents of the muscles affecting pH, such as glycogen, lactic acid, and buffers, as is needed to determine the validity of this asumption. Consideration of the relation of pH to water retention requires recognition of its complexity, specifically noting that pH is reported to affect the binding of ions to proteins and, consequently, influences water retention *(10)* and, conversely, that ion-binding may be expected to influence the buffering capacity of protein, thereby influencing pH *(28)*.

To the limited extent that zinc is mentioned in the literature concerned with water retention *(10)*, its action has been considered to be similar to that of calcium in that its binding to the structural proteins of meat has been assumed to have an adverse affect on water retention. Although zinc may, in part, have this tendency, it is parallel to pH in its relation to water retention suggesting that zinc may participate as a component, or an activator, of an enzyme the action of which may partly determine pH differentials. The formation of lactic acid from glycogen apparently varies, adding to the complexity of the system which determines pH *(19)*.

The data in Table 6 show that increasing moisture-protein ratios were directly associated with increasing water retention. As can be seen from even cursory inspection of the results given in Table 2, the moisture content of the muscles did not vary to the extent that protein content varied, producing ratios of moisture to protein ranging from 3.28 to 3.96. An extensive discussion of variation of water-to-protein ratios in different meats has been published revealing variations among cuts of meat and differences related to the age, condition, and finish of animals from which meat is obtained *(11)*. The problem in controlling the composition of sausage arising from these variations was cited. The problem would be increased, if differences in water-to-protein ratios in meats are accompanied by variation in water retention as in the case of the series of muscles presently discussed. Also, the data in Table 6 show that the tendency of increasing water retention to be accompanied by increasing fat content was marked ($r = .868 \pm .067$, $P < .01$). This would appear to be a reflection of some other factor, or factors, directly influencing water retention, inasmuch as fat itself is recognized as having little tendency to hold water. In beef fatty tissue water-to-

protein ratios ranging from 2.8 to 4.6 have been reported *(14)*.

The data in Table 6 also show that calcium, magnesium, and potassium content varied inversely with water retention, and, on the other hand, increased with increasing content of protein. The protein content and calcium, magnesium, and potassium content were found to be directly and closely related ($r = .827 \pm .087$, $P < .01$; $r = .806 \pm .097$, $P < .02$; and $r = .937 \pm .033$, $P < .01$, respectively), indicating that the content of these ions was related to protein content in relatively constant proportions. Phosphorus content also tended towards a similar relation to protein content, but the relation was significant only at the 10% level ($r = .604 \pm .175$).

The data show that iron, sodium, and chloride content tended to be directly related to water retention and to be inversely related to protein content; these relations, however, were not statistically significant, except that the content of iron was inversely related to protein content (significant at the 5% level). As previously pointed out, the correlations attempted do not indicate the independent effects of variation of single components, as has been the goal in previous work *(10, 11, 12, 30)*. The data do show that, in a series of muscles arranged in order of increasing water retention, there was found to be positive correlation with increasing pH, zinc and fat content, and ratios of moisture to protein, and negative correlation with components, including protein content (N% x 6.25), and calcium, magnesium, and potassium content.

SUMMARY

A comparative study of the composition and properties of eight different muscles from bovine animals was conducted in an investigation of factors which influence water retention. Water retention is defined as the change in the weight of the experimental meat samples after mixing the meat with distilled water (1:1) and centrifuging the meat-water mixtures for 20 minutes at 15,000 r.p.m., following overnight storage of the mixtures at 0° C.

The results indicate that considerable variation existed with respect to water retention and pH and the nitrogen, fat, moisture and electrolyte content of the muscles. The results further indicate that the relative order of the muscles of the different animals followed a pattern with regard to water retention, pH and a number of other properties.

Statistically significant correlations were obtained between water retention and directly related components, including pH, zinc and fat content, and ratios of moisture to protein, and inversely related components, including protein content (N% x 6.25), and calcium, magnesium, and potassium content.

A direct, highly significant correlation was found between water retention and zinc content, in contrast to the inverse relation found between water retention and either calcium or magnesium content. This information indicates that zinc differs in an important aspect from the two other ions. The possibility that zinc may participate in determining pH as a component of an enzyme system is pointed out.

The ratio, moisture/protein content, was found to be directly related to water retention, hence, the results show that the relative ability of the muscles to hold added moisture was predictable on the basis of the original proportions of moisture and protein present.

The analysis of factors influencing water retention is a complex problem. The present results have opened new channels for investigation; specifically, one of these concerns determination of factors influencing pH, including zinc containing enzymes, and the other, the paradoxical relation noted between protein content and water retention.

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A New Fluorometric Determination of Chlortetracycline in Ice

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Since chlortetracycline-containing ice is widely used for keeping the trawler's catches fresh in the western sea area off Japan, a practical field method for determining chlortetracycline (CTC) incorporated in ice is required to establish levels of concentration, distribution, and destruction of the antibiotic in the ice.

The present investigators reported previously on a modified acid colorimetric method for determination of CTC in ice *(11)*. This method, however, was found in some cases to give higher values for CTC than the microbiologic method does. When it was learned that the American Cyanamid Company employs ultraviolet lamps in routine field testing for detecting CTC in the use of Acronize on poultry and that the aluminum ion enhances the yellow greenish fluorescence of CTC under ultraviolet light *(1)*, a new approach was suggested. The present study was undertaken to establish a practical quantitative fluorometric method for CTC determination.

EXPERIMENTAL PROCEDURE

1. Materials and Standard Curve

A. Standard reagents

(1) A 1% potassium alum ($KAl(SO_4)_2 \cdot 12H_2O$) solution in M/2 acetate buffer of pH 4.6.

(2) M/50 citrate buffer of pH 5.2.

B. Standard solution series

(1) For laboratory Beckman spectrophotometer determination:

(a) Preparation of stock solution: Dissolve 100 mg. of CTC hydrochloride in distilled water and dilute to 200 ml. Place 1-ml. aliquots in small test tubes, stopper, freeze and store in freezer. These frozen aliquots can be preserved for 2 months.

(b) Preparation of standard solutions: To prepare standard solution series, dilute 1-ml. of stock CTC to 100 ml. with distilled water and prepare a series of test tubes containing 2, 4, 6, 8 and 10 ml., respectively. Bring the final volume of all the tubes to 10 ml. with distilled water. This standard solution series will cover a concentration range of 1 to 5 meg./ml. As these standard solutions are subject to deterioration at room temperature, it is advisable to mix them with 1 ml. of the alum reagent shortly after their preparation. The alum containing standard solution shows a consistent fluorescence for 3 hours at room temperature.

(2) For field determination: To a series of flat bottom test tubes of 27 mm. in diameter add 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml., respectively, of the diluted stock solution, and adjust volume of all the tubes to 10 ml. with distilled water. This standard solution series will cover a range of 0.5 to 5 meg./ml. Shortly after the final dilution the standard solutions shall be mixed with 1 ml. of the alum reagent. The reagent-containing standard solution can be employed for a week when kept in refrigerator as it shows only a 10% increase in the fluorescence during the storage.

2. Method of Determination

A. Preparation of sample. Place a representative 300 g. aliquot of ice sample in a beaker or polyethylene bag and thaw

by placing it in running water. Shortly after thawing add 2 ml. of M/50 citrate buffer of pH 5.2 to a 50 ml. thawed aliquot.

B. Precise determination by using Beckman spectrophotometer. After adding 1 ml. of the potassium alum reagent to a 10 ml. aliquot as directed above, allow it to stand from 20 to 60 minutes at room temperature. Measure the fluorescence of the sample at a wave length of 500 mμ which is produced with excitation light of 365 mμ. Similarly, measure the fluorescence of the other alum-containing standard solutions, subtract the blank value from each measured intensity of fluorescence, and draw a standard curve by plotting the intensity of fluorescence against the concentration of CTC. A representative standard curve is shown in Figure 5. From the standard curve read the CTC concentration of the ice sample corresponding to the intensity of fluorescence from which the blank value is subtracted.

C. Routine field estimate. Place 10 ml. of a supernatant thawed sample in a flat bottom test tube of 27 mm. in diameter, and add 1 ml. of the alum reagent. After allowing the sample to stand for 5 to 10 minutes, observe the fluorescence from the top of the tubes while irradiating with the black ray lamp from the side. Estimate CTC concentration of the ice sample by comparing the fluorescence of the sample with those of the standard series.

RESULTS

1. Metals capable of producing fluorescent CTC-chelates when irradiated with ultraviolet light. A qualitative test revealed that CTC yielded yellowish fluorescence in the presence of such metal ions as aluminum, thorium, zinc, barium, manganese, lead, strontium, tungsten, calcium and magnesium. As seen from Table 1, a comparatively strong fluorescence was observed in chelates of aluminum, thorium and zinc when the metal salts were on an equivalent ratio of 1:1 with CTC. It is to be noted that CTC showed exceedingly high fluorescence in the presence of potassium alum.

2. Fluorescence curves for metal chelates of CTC and its decomposition products. The fluorescence curve for aluminum-CTC chelate is illustrated in Figure 1. Maximum fluorescence was found at a wave length of 500 mμ. Figure 1 also shows that CTC which was inactivated by ferric ion or chlorine gave only a slight fluorescence whereas isochlortetracycline (iso-CTC) gave a noticeable intensity of fluorescence at 500 mμ. The error due to the fluorescence at 500 mμ of iso-CTC in the presence of aluminum ion can be calculated from the fluorescence curves for CTC and iso-CTC which are shown in Figure 1. Table 2 indicates that the extent of this error experimentally

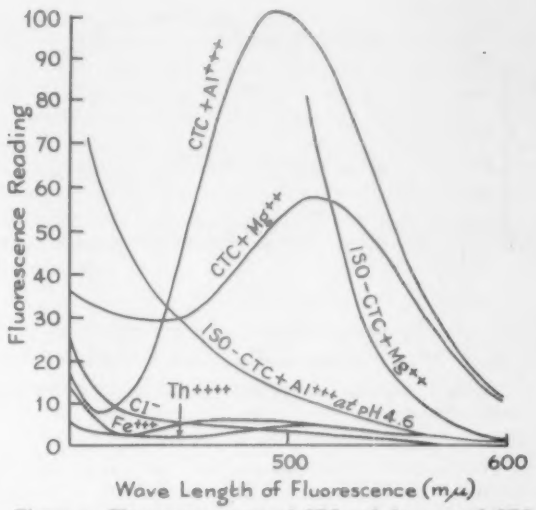

Figure 1. Fluorescence curve of CTC and decomposed CTC in the presence of aluminum ions.

obtained was close to the calculated one. This error can be neglected, however, since less than 20% of CTC in ice was found to change into iso-CTC. It is to be noted that when the excitation wave length of 436 mμ was employed, the percentage error due to the presence of 4 mcg. of iso-CTC in 1 mcg. of CTC per ml. was both calculated and found to be reduced by one-half.

3. The effect of pH on the fluorescence. The stability of metal chelate compounds is usually governed by pH value of the solution. Figure 2 illustrates that the fluorescence was stable at 4.6 but not above 5.9. The instability of the fluorescence in pH range above 5.9 seems likely to be due to deposition of the aluminum chelate above this pH value. Hence, it was decided to measure the fluorescence at pH 4.6.

4. The effect on the fluorescence of the amount of the alum reagent and time of standing after the addition of the reagent. The effect of varying the amount of the alum reagent on the fluorescence is illustrated in Figure 3. The fluorescence varied very little with increasing amounts of the reagent. In the actual determination, therefore, 1 ml. of the reagent is added for convenience to a 10-ml. aliquot of a sample solution.

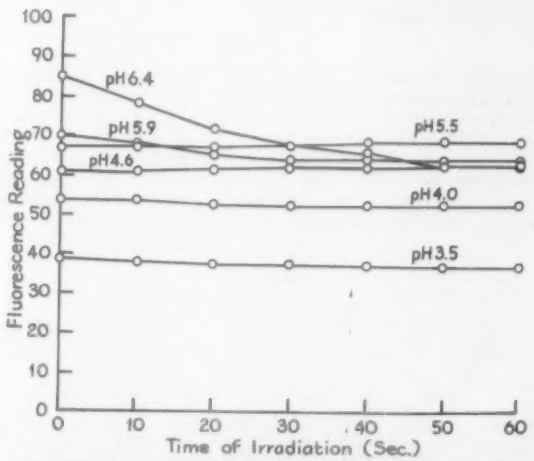

Figure 2. The effect of pH on the fluorescence of aluminum-CTC complex at 500 mμ. To 10 cc. of 5 mcg./ml. CTC in M/2 acetate buffer was added 1 ml. of 1% K Alum.

TABLE 1

Relative intensity of fluorescence of metal-CTC chelates[1]

Kinds of salt	% Concentration	Max. (mμ)	Intensity of fluorescence	
			pH 4.6	pH 6.0
$AlK(SO_4)_2 \cdot 12H_2O$	.00496	500	41 (48)	77 (26)
$Al_2O(CH_3COO)_4 \cdot 4H_2O$	.00198	500	12 (13)	15
$Th(NO_3)_4 \cdot 4H_2O$	.00580	510	12 (6)	6
$MgSO_4 \cdot 7H_2O$	.00258	510	1	2
$PbCl_2$	.00296	500	0	1
$Pb(CH_3COO)_2 \cdot 3H_2O$	.00396	500	2	2
$CaCl_2 \cdot 2H_2O$	.00154	470	1	5
$Zn(NO_3) \cdot 6H_2O$	.00353	470	6	6

[1] 10 ml. of CTC (5 mcg./ml.) in M/16 acetate buffer (pH 4.6) plus 1 ml. of metal salt solution.

Values were obtained shortly after mixing with the reagent, and values in parenthesis were those after a 20-minute standing.

TABLE 2

Interference caused by presence of iso-CTC

Sampe solution		Calculated		Found	
CTC mcg./ml.	iso-CTC mcg./ml.	CTC mcg./ml.	% error	CTC mcg./ml.	% error
4	1	4.15	3.7	4.20	5.1
3	2	3.25	8.3	3.29	9.6
2	3	2.38	19.0	2.34	16.7
1	4	1.50	50.0	1.49	49.0

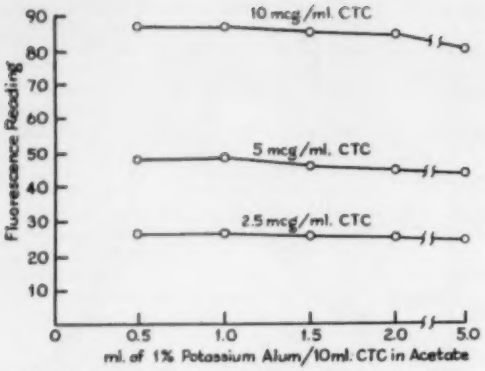

Figure 3. The effect of the alum reagent on the CTC fluorescence at 500 mμ.

The change of the fluorescence with time elapsed after the addition of the alum reagent is shown in Figure 4. It is clearly seen that the fluorescence reached a stable value after a 20-minute standing at room temperature.

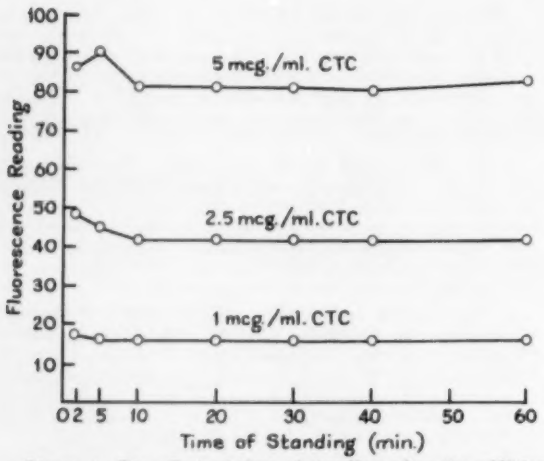

Figure 4. The effect of time of standing after the addition of the alum reagent.

5. The relationship between CTC concentration and fluorescence of aluminum-CTC chelate. Under the optimum condition described above for producing the maximum fluorescence it was demonstrated that the intensity of fluorescence of aluminum-CTC chelate at 500 mμ varied proportionately with the CTC concentration up to 8 mcg. per ml. Figure 5 illustrates this linear relationship. The regression line of the fluorescence on the concentration calculated from data given in Table 3 is

TABLE 3
Data for a standard curve

CTC mcg./ml.	Fluorescence readings					Average, ± stand. dev.	Calc.
8	100	102	103	102	107	102.8 ± 2.6	103.1
7	93.2	93.0	95.1	93.0	93.1	93.5 ± 0.9	90.7
6	76.5	75.5	77.6	76.2	76.0	76.4 ± 0.8	78.3
5	67.0	66.2	65.0	66.2	66.5	66.2 ± 0.7	65.9
4	51.3	52.0	51.0	48.0	55.0	51.5 ± 2.5	53.5
3	41.1	41.2	41.6	39.9	40.2	40.8 ± 0.7	41.1
2	29.2	30.5	29.5	30.4	30.1	29.9 ± 0.6	28.7
1	17.9	17.0	16.0	18.1	18.2	17.4 ± 0.9	16.3
0	3.3	3.3	3.8	3.0	3.6	3.4 ± 0.3	3.9

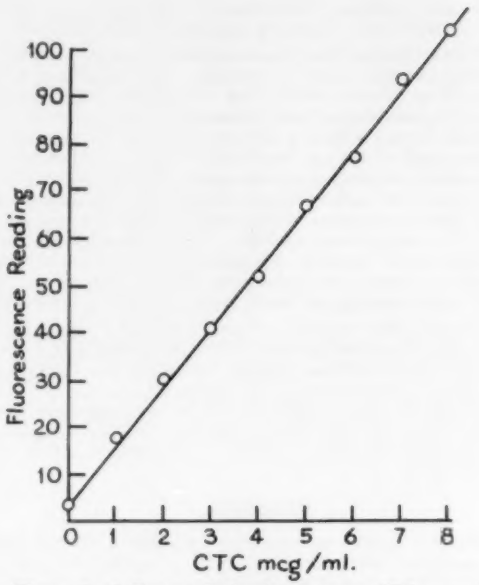

Figure 5. A linear relationship between CTC concentration and fluorescence at 500 mμ.

$y = 12.4(x - 4) \pm 53.5$ where y is the intensity of fluorescence and x, CTC concentration. The 99% confidence limits for the regression coefficient are 12.4 ± 0.35.

It was noticed that in the higher concentration range the measurement should be made shortly after mixing the sample, otherwise lower values were obtained, resulting in a discrepancy from the linear relationship.

6. Comparison between CTC values determined by microbiologic acid colorimetric, the Sakaguchi and Taguchi's, Japan FDA's and the proposed fluorometric method. Ices containing carboxymethylcellulose, CTC and sodium bisulfite or sodium thiosulfate were analyzed for CTC by various methods. The results are presented in Tables 4, 5 and 6. It will be seen that the fluorometric method yielded CTC values comparable to those respective ones assayed by the microbiologic method *(10)*. Higher values were obtained by both the acid colorimetric method *(11)* and Sakaguchi and Taguchi's method *(3)*, where-

TABLE 4
Comparison of analytical data for CTC in ice assayed by various methods

Pad plate microbiologic method mcg./g.	The fluorometric method		Acid colorimetric method	
	mcg./g.	% dev. from bioassay	mcg./g.	% dev. from bioassay
3.8	3.3	−13	4.1	+8
2.8	3.8	+36	4.6	+64
3.0	2.8	−7	3.6	+20
3.9	4.2	+8	5.1	+31
3.8	3.6	−5	4.1	+8
3.2	2.3	−28	3.3	+3
4.1	3.9	−5	5.1	+24
2.5	1.7	−32	3.5	+40
2.1	1.9	−10	5.7	+171
1.6	1.5	−6	3.4	+113
2.1	1.5	−29	3.5	+67
1.5	1.4	−7	3.1	+107
2.3	1.5	−35	3.0	+30
3.7	3.6	−3		
2.2	2.6	+18		
2.4	2.7	+13		
2.1	2.6	+24		
2.2	2.8	+27		
2.3	2.6	+13		
2.3	2.8	+22		
2.3	2.8	+22		
2.2	2.6	+18		

TABLE 5

A comparison between values for CTC in ice determined by the pad plate microbiologic method, the fluorometric method and the method of Sakaguchi and Taguchi

Microbiologic method	The fluorometric method	Sakaguchi and Taguchi's method
mcg./g.	*mcg./g.*	*mcg./g.*
2.6	1.4	4.4
2.0	1.6	4.2
1.4	1.1	5.0
1.6	1.2	2.7
3.3	2.5	5.1

TABLE 6

A comparison between values for CTC in ice determined by the pad plate microbiologic method, fluorometric method and the FDA (Japan) method

Microbiologic method	The present fluorometric method			Japan FDA method
	Spectrophotometric method	Field method, average of 4 observations	Mean dev. of 4 observations	
4.4	4.6	3.9	0.2	2.7
4.6	4.2	4.2	0.3	4.4
4.0	4.0	5.1	0.1	4.0
4.6	4.0	3.8	0.2	4.1
3.3	4.1	3.4	0.2	1.8
1.0	2.5	2.6	0.2	1.8
0.8	2.0	1.5	0.2	1.3
1.0	1.8	1.6	0.1	0.5
1.4	1.0	1.3	0.1	1.1
4.7	4.3	4.0	0.2	2.3
4.7	4.7	4.4	0.3	2.3
4.3	4.6	4.7	0.4	2.4
3.9	4.4	4.3	0.3	2.3
2.2	3.6	2.9	0.1	3.5
2.5	3.4	3.5	0.1	2.5

as lower values were generally obtained by the FDA (Japan) procedure. The latter method is a modification of fluorometric method for iso-CTC determination in which the measurement is made after a 15-minute standing of a mixture of 95 ml. of sample and 5 ml. of 5N NaOH.

Data in Table 6 indicate that a simple visual comparison between the sample and the standard series yielded values comparable to the respective ones measured fluorometrically by the Beckman spectrophotometer.

DISCUSSION

Ishidate and Sakaguchi *(2)* and Sakaguchi *et al. (4–8)* made extensive studies on metal-CTC chelates. Sakaguchi and Taguchi *(3, 9)* made use of the absorbance of thorium-CTC chelates at 400 mμ for the determination of CTC in aqueous solution as well as in biological materials. It has been found, however, that the yellow fluorescence herein reported is more sensitive to low CTC concentrations than is the yellow coloration of thorium chelate. It has been further demonstrated that Sakaguchi and Taguchi's method is not appropriate for CTC assay in ice since values obtained by their method are always higher than those by the microbiologic method.

The widely used fluorometric method for the determination of CTC employs the blue fluorescence of alkali-treated CTC, i.e., iso-CTC. So far as the authors are aware, no attempt has been made to utilize the fluorescence of the metal chelate for a chemical assay of CTC.

One of the advantages of the proposed method is that the fluorescence of aluminum-CTC chelate is not affected by the presence of decomposition products of CTC, nor by turbid substances in the sample. The feasibility of the fluorometric method for CTC assay in ice is evidenced by data indicating a fairly good agreement between values for CTC in ice as assayed by the fluorometric method and microbiologic method as contrasted to the other methods. Another advantage is that the procedure is simple enough to permit field assay for CTC without special laboratory facilities. The method is sufficiently precise as a field estimate of CTC since it can differentiate as small a difference as 0.5 mcg. per ml. by a simple visual comparison.

SUMMARY

A new fluorometric method for determining CTC in ice or in solution has been presented. The method was established by data showing a linear relationship between CTC concentration and intensity of fluorescence of aluminum-CTC chelate and also little interference due to presence of decomposition products of CTC. A fairly good agreement was obtained between CTC values as assayed by the microbiologic method and the proposed fluorometric method. This method can be employed for field assays of CTC concentration in ice.

Acknowledgment

The authors are very grateful to American Cyanamid Co. for a grant-in-aid which made this work possible.

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Central Station Supervisory Control for the Food Processing Industry*

(Manuscript received May 31, 1958)

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Taylor Instrument Companies, Oak Park, Illinois

IT IS TIMELY to suggest to technologists that they take a more active and aggressive role in the instrumentation of food processing plants. It could well be that a general up-grading of plant efficiency, quality, and yield would result. Food technologists are currently active in specifying and determining and improving the "production environment" of food processing, thereby insuring quality and general acceptance of food products. They have a voice in choosing processing equipment, and they advise on procedures and ingredients. As a result, the food processing plants over the nation have produced more saleable products. A similar, vigorous attitude toward instrumentation would result in well engineered control systems of real economic value to the food processing industry.

There is a tendency to deal with process instrumentation in the food industry in a rather vague and incomplete manner, rather than to seek to fully integrate all phases into a homogeneous system. By contrast, the chemical and petroleum technologists take an active part in determining instrumentation. It is not uncommon for the chemical and petroleum industry to spend from 5 to 10% of their plant expenditures on instrumentation, and percentage-wise the time allotted to study all phases of the instrumentation is extremely high. Since the yield and quality of the product is closely linked to the accuracy of his judgment, it is imperative that the chemical (or petroleum) technologist have proper tools at his disposal. The food technologist has a comparable role and an equally vital interest in product quality and yield.

ENVIRONMENTAL CONTROL SYSTEMS

Most food processing plants accept and extensively use an environmental control system. Typical locally mounted controls are illustrated in Figures 1 and 2. Usually, the controller is adjacent to the equipment being controlled, even though a complex coordinated system is involved (the term co-ordinated control designates a combination of several control elements into a single unified system designed to make a unit process operation completely automatic). Figure 1 illustrates a coordinated retorting system; the operator's responsibility is limited to loading the retort, closing the lid and removing the basket. The illustrated system interchangeably handles tin and glass processing, automatically sequencing and controlling all necessary variables, such as venting, processing temperature and pressure, timing and cooling. Electrical interlocks are so arranged that unless a previous requirement, time, temperature, etc., are satisfied, the next sequence can not be initiated. The key advantages, of course, are uniformity and repeatability in reference to the processed product regardless of external service variations.

Figure 1. Locally mounted controls: a coordinated retorting system.

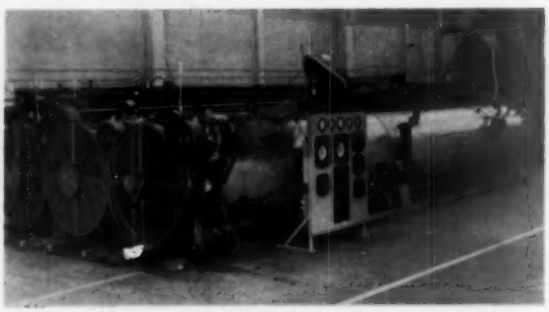

Figure 2. Controls mounted adjacent to the cooker, a further example of locally mounted controls.

Figure 2 illustrates a cooker with all instrumentation and controls mounted on a panel just adjacent to the cooker itself. This control system can run unattended until some off-normal condition exists which will allow alarm control circuits to cause a fail safe condition. Other examples of locally mounted control systems or blanchers, heat exchangers, or brine density control on a pea grader could also be illustrated.

These figures all illustrate the general acceptance of instrumentation. However, they fall into the category of sub-station control and obviously, if the co-ordination of these and any other pieces of equipment are necessary, the adjustments of controllers (as required) must be made in the plant area in which the equipment is located.

* Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, Illinois, May 28, 1958.

CENTRAL STATION CONTROL

Many years ago, the chemical and petroleum industries used this general approach to instrumentation, but have abandoned the sub-station type control, and are now using almost exclusively a central control room to check, adjust, and optimalize all phases of their production. The supervisory control or central station control approach to instrumentation is recognized as a "must" in these industries.

These systems, originally a series of unit processes, have been integrated into a continuous operation. Most food processing plants can be modified in a similar fashion to permit a continuous controlled operation.

Figure 3 illustrates a typical control board in a petroleum refinery or chemical plant where most of the environmental control adjustments, production rates, etc., are adjusted from this central control panel board. The responsibility for quality and production is in the hands of a small group of highly trained operators. The supervision of all phases of this process is co-ordinated at this nerve center. Figure 3 shows a central control board; the actual diagram of the processes, controls, and control valves are placed in the panel board. The variables that are controlled or measured are placed pictorially where they are measured or controlled. These plants have a common problem with most food processing plants. "Mother Nature," in producing the raw materials, does not produce uniformly. Consequently, decisions are constantly being made to alter the environmental variables being controlled so that optimum production results. Speed in making these decisions and the ability to adjust the variables under control make it possible to minimize any losses and reduce blending or re-runs necessary in producing quality product.

Figure 3. A typical control board in a petroleum refinery or chemical plant. Environmental control adjustments derive from this "nerve center."

Making adjustments. The convenience of making adjustments is very important in these plants; with high production rates, the duration of off-normal conditions is directly related to the cost of production. Certainly the food processing plant has a similar requirement for acting rapidly and wisely because they do not always have the opportunity of making blends or re-running the product as the petroleum or chemical plant does. Actually, the food processing plant has a greater risk because if the food is being run off-normal, it could be either a total loss or downgraded to the extent that profit is lowered or lost entirely.

Mentally picture your own plant with local control systems in the 4 corners of the factory. Now visualize the time when off-normal conditions exist and relate into the minutes the time it takes for your supervisors to assemble, confer, make decisions, and act in order to get the plant running smoothly again as an integrated unit.

Mentally bring the instrumentation to one central panel board; variables can now be observed at this panel board without a plant tour. Therefore, the time lag, to understand the real problem, is reduced, and immediate optimalizing adjustments are possible.

CANNING LINE CONTROL

Figure 4 illustrates the graphic approach to a simple canning line with three sets of blancher and quality grader controls, and one Food Machinery and Chemical Corporation continuous cooker control.

Figure 5 illustrates a central station panel board which is one of the first, if not the first, to attempt to centralize the control of the canning plant.

Figure 5 is an installation at Libby, McNeill and Libby in Wallaceburg, Ontario, Canada. The panel represents a large percentage of the instruments used in processing a variety of vegetables. The upper center portion of this panel board has nine miniature recording controllers. Immediately below the recorders are indicating controllers. Below the indicators are a series of lights which indicate hopper levels or show the operator which pump or other piece of processing equipment is operating. On the extreme right of the control panel are counters for tabulating the total production of corn cutters and huskers. The three round dials on the left hand side of the panel board indicate to the operator the speed of the feed elevators and immediately below each of these dials are the devices for adjusting the speed or "stopping and starting" the elevators.

The actual instrumentation involved here is not too different from the sub-station type of control except that the panel board and the measuring devices are separated by electric wire or copper tubing. The field instrumentation consists of transmitters as illustrated in Figure 6. These measuring elements transmit signals to the central station control board; the recorders and controller at the control board operate control valves at processing equipment location. These transmitters permit narrower ranges than are now available with conventional type instruments. Range

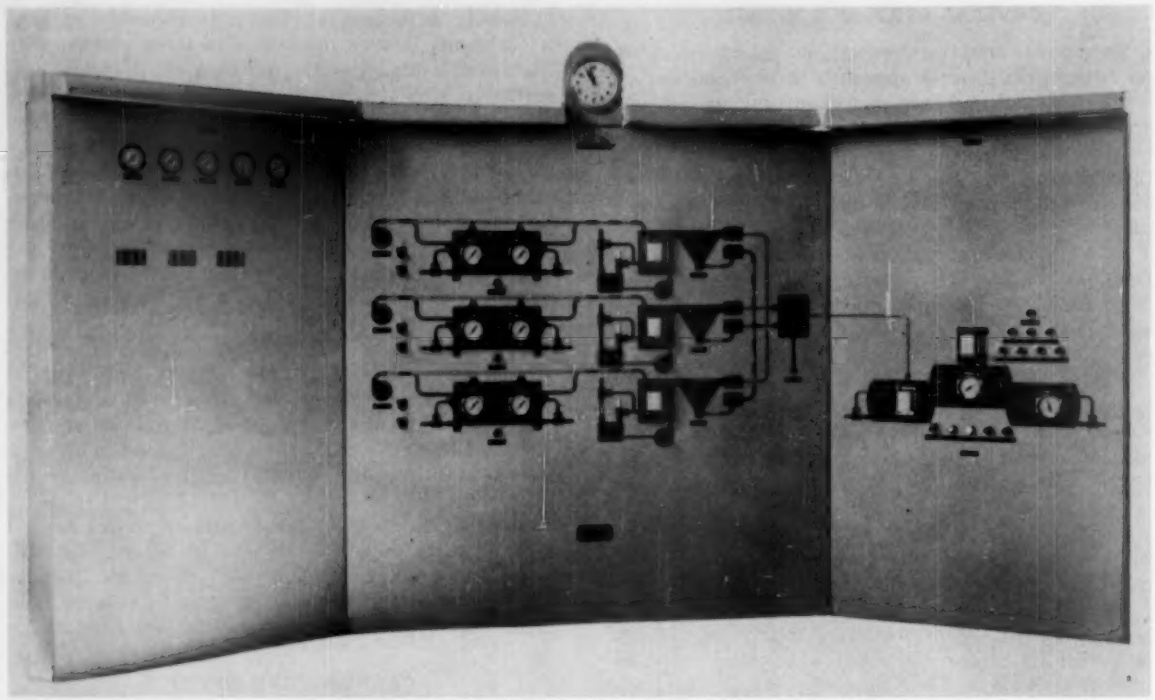

Figure 4. A simple canning line control for a continuous cooker.

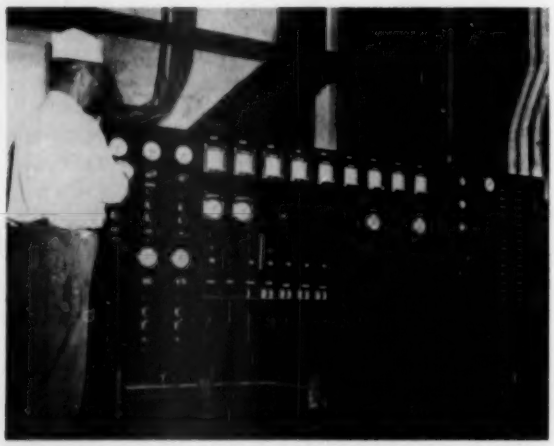

Figure 5. An installation at Libby, McNeill and Libby, Wallaceburg, Ontario.

span adjustments are made with a screwdriver, making the instrument more versatile. Since these devices are small and lightweight, they are easily shifted from one piece of equipment to another. Therefore, these instruments are multi-purpose devices. Several of the instruments on this panel board as shown in Figure 5 illustrate this multiple purpose. Actually, the top row of recording controllers are used for control on several processes. The same recorder on this board can be used as a control mechanism during the procesing of peas and later used during the corn season or pumpkin season with only minor changes.

Regarding the remote adjustment of the feed elevator, a diaphragm motor operates the variable speed drive and the operator at the control board can stop and start the motor; a simple adjustment of the reducing valve at the panel board will give him an infinite number of speeds between 0 to 100% of maximum speed.

Many of the local control systems applied to specific stations in this plant are such that they are virtually unattended except for the supervising operator at the panel.

At other stations, the processing is either controlled, stopped or started depending upon a preceding operation or supply condition. Obviously, this nerve center permits correlation at one point of vital information

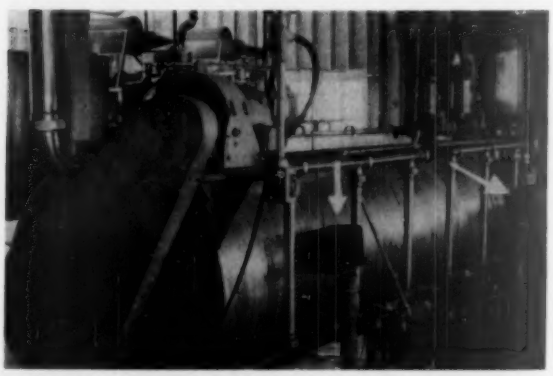

Figure 6. Measuring elements transmit signals to central station control board from processing equipment location.

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376A

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(Reg. U. S. Pat. Office)

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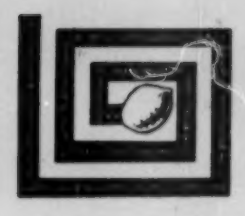

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which heretofore was spread out to the four corners of the canning plant. An operator at this board can remotely either manually or automatically control all of the variables which determine the environment under which the product is processed. This particular control board is being used to process a variety of vegetables which are not necessarily processed simultaneously and therefore, many of the recording and controlling devices have multiple purpose. An alarm system alerts the operator when something is "off-normal." The operator can then quickly take corrective steps if equipment has failed. By telephone or by close proximity of raw material storage, etc., the operator can decide what processing variables need to be altered. Minimum losses and maximum yield are attainable with such a nerve center, and it is safe to conclude that a loss of product from the overflowing of hoppers because of temporary bottlenecks has been eliminated. Certainly within safety limitation, other controls and devices can be added to this control board until the "push-button" food processing plant is virtually a reality.

ADVANTAGES OF CONTINUOUS PRODUCT ANALYSIS BY INSTRUMENTATION

Continuous product analysis instrumentation for qualitative determination (some of which has been described) added to central station control panel boards would be a very distinct advantage to the supervisor in making swift decisions.

Manpower reduction may or may not result from central station supervisory control. This would vary from plant to plant, but in comparing this central station concept to the sub-station control concept, without a manpower reduction, it may be economically justified by reduction of losses. One good technical man can run your plant from the panel board. Maintenance and preparation responsibilities are shifted to others (possibly seasonal employees).

The operation will be more efficient because responsibility is centralized. All pertinent information is visible at the panel board. Temporary equipment failure will necessitate action on the part of the operator to divert the product, speed up parallel equipment or start standby equipment so that production can continue without serious loss. If the quality of the product is determined by changing environmental conditions when raw materials vary, then certainly quick adjustment of the controls can easily be made at this type of panel board, and this responsibility is placed in the technologist's capable hands. The inconvenience of someone going to a sub-station controller at a remote place in the plant to adjust the instrument is therefore eliminated, and possible errors are minimized.

During this discussion, repeated references to the chemical and petroleum industries' experience have been made. Data logging devices and computers are in their infancy for these industries, and it could very well be that the food industry will find economic advantage to such techniques also. It should be pointed out that the plant we have discussed here has merely adapted continuous processing techniques to their specific requirements. All this has been accomplished without a materially higher initial installation cost.

The really important facet of the central station control concept is the co-ordination of all phases of processing at one point where it is possible for one individual or a group of individuals to make rapid and accurate decisions regarding production and quality. This means lower losses and higher profits.

Bacteriological Flora of Frozen Coconut

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(Manuscript received November 14, 1958)

IN CERTAIN INSTANCES coconut meat is marketed in the frozen state as a coconut-sucrose mixture (3 parts coconut, 1 part sucrose). Preliminary findings from primary plating of frozen coconut-sucrose samples on desoxycholate lactose agar yielded bacterial colonies characteristic of coliform types. A literature search failed to reveal any published data on the microbial flora of coconuts and it seemed pertinent to extend the original findings with additional taxonomic studies.

EXPERIMENTAL

The coconut samples investigated were either frozen shaved nuts or frozen coconut-sucrose mixtures obtained from commercial sources; or research samples processed according to commercial procedures. Serial dilutions of samples were plated in desoxycholate lactose agar supplemented with 0.1% sucrose. The supplement was added because quality control evaluation of samples of this type require consideration of the presence of sucrose. Usually lactose broth cultures were prepared from typical coliform colonies and stock isolates were maintained on nutrient agar. One series of cultures, obtained from M. Purko,[a] was repurified from eosin methylene blue agar.

Pathogenicity for carrot tissue was determined by the method of Elrod *(3)* modified to include a 30 second chlorine wash (200 p.p.m.) of the tissue, followed by a sterile distilled water wash. This modification yielded a higher percentage of sterile carrot-broth tubes. Gram stains, acetylmethylcarbinol and methyl red tests as well as the utilization of citrate were determined according to standard procedures *(6)*. Hydrogen sulfide production was determined by the method of Levine *et al. (4)*. Control cultures (*Escherichia coli* ATCC 11229 and our strain CO-36; *Aerobacter aerogenes* NRRL 199 and our strain CO-39) were tested concurrently with unknown cultures.

RESULTS AND DISCUSSION

Preliminary investigations with typical isolates from desoxycholate lactose agar, indicated that some of these organisms were false-positive types dependent upon the presence of sucrose or other fermentable non-lactose carbohydrate in the sample. In the present study 121 bacterial cultures were more thoroughly characterized. Microscopic observations showed all isolates to consist of gram-negative nonsporeforming rod types. On the basis of exhibiting pathogenicity for carrot tissue (soft-rot), some of these isolates could be classified as representing the genus *Erwinia (3)*.

Furthermore a substantial number of cultures could be eliminated from the coliform group by their inability to produce gas from lactose *(2)*. These findings are presented in Table 1. Approximately one-third of the original isolates were true coliform bacteria.

Further classification of the coliform types was made on the basis of responses to indole, methyl red and acethymethylcarbinol (Voges-Proskauer) tests, plus citrate utilization. The taxonomic grouping of these organisms according to the criteria of Vaughn and Levine *(7)* is shown in Table 2. This classification scheme indicated the presence of only a single *Escherichia* strain although 15% of the total isolates were not classifiable by this scheme. Tabulation according to the scheme of Parr *(5)* is presented in Table 3. A single culture was identifiable as *Escherichia coli* with about equal numbers of *Aerobacter aerogenes* and "Intermediate" types. These latter two types are not considered to be of fecal origin and therefore are not indicative of fecal contamination *(1)*.

TABLE 1

Classification of gram-negative nonspore-forming rod isolates from coconut samples. 121 isolates

Classification	Distinguishing reaction	Per cent of total isolates	Reference
Erwinia	Carrot (soft rot) +	16	Elrod, 1942
non *Erwinia*	Carrot (soft rot) —	84	Elrod, 1942
coliform	Lactose (gas) +	36	Breed *et al.*, 1948
noncoliform	Lactose (gas) —	48	Breed *et al.*, 1948

TABLE 2

Coconut coliform bacteria classified according to Vaughn and Levine, 1942

Classification	Distinguishing reactions[1]			Per cent of total isolates	Per cent of coliform isolates
	V.P.	M.R.	Cit.		
Escherichia	—	+	—	<1	2 (single isolate)
"Intermediate"	—	+	+	15	41
Aerobacter	+	—	+	6	16
Unclassified	—	—	+	15	41

[1] V.P.—Voges-Proskauer. M.R.—Methyl Red. Cit.—Citrate utilization.

TABLE 3

Coconut coliform bacteria classified according to Parr (1939)

Classification	Distinguishing reactions[1]				Per cent of total isolates	Per cent of coliform isolates
	I.	M.R.	V.P	Cit.		
E. coli	—	+	—	—	<1	2 (single isolate)
"Intermediate"	—	+	—	+	12	32
	+	+	—	+	3	9
	+	—	—	+	2	5
					17	46
A. aerogenes	—	—	—	+	13	36
	—	—	+	+	6	16
					19	52

[1] I.—Indole. M.R.—Methyl Red. V.P.—Voges Proskauer. Cit.—Citrate utilization.

SUMMARY

Ninety-nine per cent of the positive reactions obtained by conventional coliform determinations on

[a] Research and Development Division, National Dairy Products Corporation, Glenview, Ill.

fresh coconut were false-positive reactions for fecal coliforms. Two reasons are evident for the occurrence of these reactions: First, the presence of fermentable carbohydrate other than lactose; second, the presence of lactose fermenting organisms which are not true indicators of fecal contamination. About 15% of the false reactions appeared to be due to organisms which are pathogenic for plants.

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Measurement of Preferences for Food Combinations[a, b]

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One of the best ways of predicting whether people will eat a food, or how much of it they will eat, is to ask them how well they like it. The validity of this assertion is amply supported by the results of several field studies where correlations on the order of .70 were found between consumption and preference rating of food names *(2)*. However, prediction might be improved by evaluating certain additional facets of food behavior. One salient aspect of eating is that foods are usually eaten together as a meal, rather than singly. Does this practice of eating foods in combination affect food attitudes?

Combinations may be defined as several foods, individually prepared, that are served and eaten either together or successively. (Blended foods, such as stews, are excluded from this definition.) It is hypothesized that when two foods are combined in this manner, the individual components partially lose their identity in favor of a new entity, the combination, with its own unique associated preference. Thus preferences for the individual components may be non-additive, i.e., preference for the combination might not be predictable from any sum or weighted average of preferences for the components. For example, a person may only slightly like each of two foods by themselves, but he may like their combination very much.

This hypothesis of combination effects is in part supported by evidence from several pilot studies run in conjunction with attitude surveys conducted among military personnel. These studies also provided information on the suitability and limitations of two types of questionnaires for measuring preference for food combinations. One questionnaire presented the respondent with an entire menu to be rated on a single scale. Judging the combination

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[b] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 883 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

effects of a whole menu may have been too difficult a task for respondents; their ratings appeared to reflect preference for the main dish rather than for the menu as a whole. The second questionnaire required respondents to select from a list of food items that one food which in combination with a given food would make the best possible pair. This questionnaire indicated non-additivity for meat-potato combinations, but not for meat-vegetable combinations. There were inherent limitations in this questionnaire. Certain items on the list were chosen at much higher frequencies than others, resulting in little information for the items not chosen, and selection of an item was relative to the remaining items in the list. A review of the psychological literature showed that other than these investigations there have been few studies of combined stimuli in any sensory modality, and none specifically on foods.

Combination effects have important implications for menu planning, e.g., determining the best combinations for foods of low individual preference. Moreover, in situations such as institutional feeding, where menus must be composed of a limited number of foods, the greatest number of satisfactory combinations which the components may yield can be determined.

The purposes of the pilot study reported here were to obtain substantive information on attitudes towards food combinations, and to determine the suitability of a questionnaire method designed to measure such attitudes.

METHODOLOGY

Two considerations appeared important in designing the combination questionnaire: simplifying the respondent's task, and providing equal amounts of information for each combination. The former was met by presenting the respondent with only 2 foods at a time, reserving combinations of more than 2 foods for future research. The second objective was achieved by having each food combination in a questionnaire rated by every respondent.

The basic format of the questionnaire consisted of combination blocks formed by a key food followed by a list of 5 sub-items. The example below shows the key food, 2 sub-items, and the 7-point rating scale.

Two combination blocks were placed on each page of the questionnaire. The respondent's task was to rate the combination of each key food with each sub-item on the adjacent scale.

This study was restricted to 3 food classes: main dishes, potatoes, and vegetables. The key foods were selected from the main dish or potato classes; sub-items were taken from the potato or vegetable classes. Only one potato sub-item list, consisting of 5 potato dishes, was used. All vegetable combinations also employed one vegetable sub-item list. Main dishes were paired with either the potato or vegetable sub-items list, but when the key item was a potato dish, only vegetable sub-items were used. The specific foods selected as key or sub-items had a wide range of individual preferences as determined by previous surveys of single foods.

How well do FRENCH FRIED POTATOES go with

Buttered green peas.....	extremely well	very well	moderately well	slightly well	slightly poor	very poor	extremely poor	cannot decide
Sauerkraut.....	extremely well	very well	moderately well	slightly well	slightly poor	very poor	extremely poor	cannot decide

Six forms of this basic questionnaire encompassed the variables to be explored. Forms 1, 2, and 3 employed the same format but had different key foods in order to produce a greater total number of combinations. Positions of combinations of specific food classes were also varied among the 3 forms, so that meat-potato combinations did not always appear first. Each of these forms had 10 key foods, making a total of 50 combinations. The remaining 3 forms were identical to Form 1, except for certain planned variations. The only difference in Form 4 was that it lacked the *cannot decide* category present on all other forms. Forms 5 and 6 each contained 50 food combinations in addition to the 50 used in Form 1. The 50 extra combinations served as "padding" in order to test the effects of doubling questionnaire length. Sub-item lists were lengthened in Form 5 and the key item list was doubled in Form 6. The "padding" combinations always preceded the combinations included in Form 1. Forms 4, 5, and 6 were analyzed by comparing the ratings for those combinations common to Form 1.

ADMINISTRATION OF QUESTIONNAIRES

A total of 593 respondents, selected from Army training units at Fort Lee, Virginia, answered the questionnaires at one of 5 sessions. The respondents had a higher educational level than is generally found for randomly selected Army samples. Thirty-two questionnaires reflecting careless responses or misunderstanding were rejected, leaving 90 or more useable questionnaires of each form for analysis. Three testing rooms were used in order to eliminate interference arising from differences in form length or instructions. Following the administration of the combination questionnaire, the respondents rated the individual foods on a hedonic scale questionnaire *(1)*. In addition, at the conclusion of the test, a small sample of the respondents were interviewed about the questionnaire and the food combinations.

DATA PROCESSING

For analysis, the scale categories in the combination questionnaires were assigned successive integers from 1 to 7 and in the hedonic preference questionnaire, integers from 1 to 9. In both cases "1" corresponded to the scale category reflecting the most unfavorable attitude. Ratings of every combination were summed by form and session for an analysis of variance of combination effects. General preference was found by averaging all ratings for a combination in a given form. Preferences for individual foods were obtained by averaging ratings of all respondents.

RESULTS AND DISCUSSION

The mean ratings for the different combinations appearing in the first 3 questionnaire forms are shown in Table 1. The combinations are arranged by food class in two-way tables. The key foods are listed in the column in order of descending preference as determined on the hedonic scale questionnaire. The sub-item foods are shown as column headings, with the individual preference rating appearing immediately below each name. Combination effects are shown for various foods; for example, baked ham has a higher rating when paired with candied sweet potatoes or mashed potatoes than when paired with french fries. If strict additivity had prevailed, baked ham

TABLE 1

Mean combination ratings for Forms 1, 2, and 3

Key foods	Individual preference ratings*	Sub items				
		French fried potato	Mashed potato	Potato salad	Baked potato	Candied sweet potato
a. Main dish—potato combinations		7.96*	7.83*	7.28*	6.63*	6.62*
Roast turkey	8.22	4.95	6.76	4.83	5.16	5.80
Roast chicken	7.99	5.42	6.51	4.71	4.91	4.87
Baked ham	7.77	5.26	5.97	5.61	5.48	5.97
Roast pork	7.40	4.98	5.89	4.20	4.70	4.45
Frankfurters	6.38	5.63	4.16	5.48	4.41	3.30
Corned beef	6.19	5.08	5.07	4.30	4.89	4.17
Beef stew	6.02	4.00	4.04	3.22	4.37	2.83
Fried fish	5.88	5.58	3.97	4.38	4.28	2.75
Salmon cakes	5.79	5.01	4.87	4.37	4.86	3.71
Chop suey	5.48	3.67	3.26	3.18	3.43	2.80
Grilled liver	5.33	5.04	5.09	3.66	4.52	3.75
Lamb stew	5.14	3.89	4.69	3.62	4.43	3.49
		Fresh sl. tomato	Creamed corn	Buttered gr. peas	Sauer-kraut	Buttered cauli-flower
b. Main dish—vegetable combinations		7.86*	7.21*	7.05*	5.12*	4.74*
Roast turkey	8.22	5.37	5.71	5.96	2.30	4.08
Roast beef	8.08	5.34	5.33	5.75	2.72	4.11
Roast chicken	7.99	5.02	5.83	5.71	1.99	3.80
Baked ham	7.77	5.33	5.41	5.40	3.57	4.17
Roast pork	7.40	5.39	5.39	5.77	3.95	4.00
Corned beef	6.19	4.87	4.92	4.68	3.40	3.67
Beef stew	6.02	4.77	4.37	4.72	3.78	3.67
Fried fish	5.88	4.92	4.41	4.65	2.45	3.32
Grilled liver	5.33	4.70	4.87	4.64	2.54	3.49
Lamb stew	5.14	4.39	4.68	4.20	2.72	3.65
c. Potato—vegetable combinations						
French fried	7.96	5.63	5.13	5.01	3.11	3.61
Mashed	7.83	5.31	5.77	5.79	3.60	4.12
Potato salad	7.28	5.92	4.47	4.18	2.83	3.46
Baked	6.63	5.37	5.51	5.72	3.41	3.97
Candied sweet	6.62	4.70	4.67	4.56	2.61	3.35
Baked sweet	6.58	4.55	4.90	4.67	2.60	3.57
Hash browned	6.39	5.51	5.51	5.49	3.23	4.11
Boiled	6.24	5.02	5.49	5.27	3.60	4.19
Scalloped	5.94	4.79	4.91	4.80	2.76	3.61

* Individual mean preference ratings (9-point hedonic scale).

should have the highest combination rating with french fries and the lowest with candied sweet potatoes. Non-additivity for other food items is discernible when the combination rating is compared to the corresponding individual preference rating. The most marked combination effects occurred in the main dish-potato combinations. For potatoes and vegetables the magnitude of these effects was smaller.

Food combination effects are demonstrated when the food interaction term of an analysis of variance of ratings is shown to be significant. Separate analyses of variance were done on Forms 2 and 3 for each set of combinations and are presented in Tables 2 and 3. Significant interactions were found in each form for main dish-potato, and main dish-vegetable combinations, but were not obtained for the potato-vegetable combinations. The significant differences for the main effects of meats, potatoes, and vegetables were expected inasmuch as the components were selected to have a wide range of preference. In some cases significant session effects were found, indicating differences among respondents, but these differences do not affect the general conclusions regarding combinations.

Food and questionnaire form effects were investigated by similar analyses of variance (not shown) on Short Forms 1 and 4, and Long Forms 5 and 6. These 4 forms had identical food combinations, which differed from those included in Forms 2 and 3. The different types of food combinations were analyzed separately with results generally similar to those in the preceding analysis. Significant interactions were found for the main dish-potato and main dish-vegetable combinations. However, here, a small but significant interaction was found for potato-vegetable combinations. This may have been due to the fact that different items were used in these combinations.

With regard to form, the absolute level of rating differed between long and short forms for all except potato-vegetable combinations. Despite these significant main effects, form had little or no effect on the relative combination ratings, as shown by the low form-combination interactions. Equivalent combination results between the different forms suggests that as many as 100 combinations may be included in a questionnaire; however, this conclusion may not be generalizable to a population having a lower educational status.

On Form 4, which lacked the *cannot decide* category, there were 131 *omits* (no response), compared to 26 on Form 1. A separate analysis showed that the difference in number of *omits* between forms over sessions was significant at the 5% level. Inasmuch as

TABLE 2

Analysis of variance of food combinations included in Form 2

Variable	df	Mean square	"F"	P
a. Main dish—potato combination				
Session	4	.64	7.08	.001
Main dish	3	3.17	34.85	.001
Potatoes	4	5.04	55.34	.001
Sess.—Main dish	12	.30	3.30	.001
Sess.—Potatoes	16	.16	1.74	
Main dish—Potatoes	12	1.30	14.23	.001
Sess.—Main dish—Potatoes	48	.09		
Total	99			
b. Main dish—vegetable combination				
Session	4	.10	1.03	
Main dish	2	6.16	66.46	.001
Vegetable	4	13.27	143.18	.001
Sess.—Main dish	8	.14	1.49	
Sess.—Vegetable	16	.58	6.24	.001
Main dish—Vegetable	8	.27	2.95	.01
Sess.—Main dish—Veg.	32	.09		
Total	74			
c. Potato—vegetable combination				
Session	4	.73	3.10	.05
Potatoes	2	1.46	6.24	.01
Vegetable	4	15.89	67.79	.001
Sess.—Potatoes	8	.20	<1.	
Sess.—Vegetable	16	.16	<1.	
Potatoes-Vegetable	8	.20	<1.	
Sess.—Potatoes-Vegetable	32	.23		
Total	74			

TABLE 3

Analysis of variance of food combinations included in Form 3

Variable	df	Mean square	"F"	P
a. Main dish—potato combination				
Session	4	.30	2.05	
Main dish	3	14.73	102.05	.001
Potatoes	4	2.00	13.84	.001
Sess.—Main dish	12	.13	<1.	
Sess.—Potatoes	16	.34	2.38	.01
Main dish—Potatoes	12	1.74	12.09	.001
Sess.—Main dish—Potatoes	48	.14		
Total	99			
b. Main dish—vegetable combination				
Session	4	.07	<1.	
Main dish	2	1.16	9.30	.001
Vegetable	4	16.91	135.36	.001
Sess.—Main dish	8	.29	2.29	.05
Sess.—Vegetable	16	.72	5.75	.001
Main dish—Vegetable	8	1.29	10.33	.001
Sess.—Main dish—Veg.	32	.12		
Total	74			
c. Potato—vegetable combination				
Session	4	.16	2.21	
Potatoes	2	4.48	61.23	.001
Vegetable	4	15.63	213.78	.001
Sess.—Potatoes	8	.13	1.80	
Sess.—Vegetable	16	.78	10.65	.001
Potatoes-Vegetable	8	.09	1.29	
Sess.—Potatoes-Vegetable	32	.07		
Total	74			

endorsements of the *cannot decide* category on Form 1 were approximately equal to the number omitted on Form 4, it appears that *omits* and *cannot decide* fulfill the same purpose. Presence or absence of the *cannot decide* category did not affect combination ratings.

RESPONDENT INTERVIEWS

Supplementary to the major objectives of this study, small randomly selected samples (16%) of the respondents were personally interviewed at the termination of each session. Six open-end questions were used as a guide. Eighty-five per cent of the respondents found rating the combinations more difficult than rating individual foods. Sixty-two per cent of interviewees stated that they rated on the basis of the combination, whereas 19% and 12% indicated their responses were more influenced by key items and sub-items, respectively. Almost half of the respondents said that the main dish-potato combinations were easiest to rate; the remainder indicated main dish-vegetable combinations were easiest. Reasons most often given for ease of rating were liking for the particular combination and fairly frequent past experience with it. Asked how they felt about combinations, 86% said that food combinations were important to them. This evidence indicates that the respondents were, in fact, considering combinations rather than the component foods.

SUMMARY

The hypothesis that preference for a food combination differs from the sum or weighted average of the individual component preferences was investigated in a pilot study. Questionnaires requiring ratings on how well the foods of a pair go together were completed by 593 military personnel. One important variable studied was form length; forms containing 50 or 100 combinations were used. Three food classes were used for the combinations: meats, potatoes, and vegetables.

Results of the study indicated that how well main dishes and potatoes go together, and how well main dishes and vegetables go together, are in large part independent of preferences for the individual components. Potato-vegetable combination effects were not generally found, but this may have resulted from the specific foods paired. Differences between forms generally had little or no effect on the food combination effects.

The investigation of combination preferences by a questionnaire method was shown to be feasible.

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The Influence of High Temperature Holding Upon the Components of Coffee Brew.

II. Volatile Reducing Substances[a]

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ONE OF THE ATTRACTIVE qualities of a cup of coffee is the fine aroma produced upon its preparation. It has been the desire of coffee producers and users to capture this aroma in the beverage itself. The production of an instant-type coffee, which produces a cup with the aroma of coffee freshly prepared from coffee beans, has long been the goal of the coffee industry. A close adjunct to this problem has been that of finding a reliable means for the objective evaluation of coffee aroma and flavor.

Some objective means of evaluating the effect of various treatments on the volatile flavor of coffee would be of benefit to investigators and commercial operators alike. Hogl *(2)* described a method for the evaluation of aroma by means of an examination of the steam distillate of coffee. He stated that the amount of material which was oxidized by acid dichromate in a sample of the steam distillate from one gram of coffee was the "aroma value" of that coffee and that these results were "in fair agreement with those obtained by organoleptic tests." Wurtziger *(7)* carried out extensive studies based on this method. He modified and simplified the apparatus of Hogl but used the same basic method. He used the number of ml. of 0.1 *N* dichromate necessary to oxidize the steam distillable materials from a one gram sample of coffee as an indication of the "oxidation value."

Ehlers *(1)* considered that the aroma values obtained by Wurtziger from the titration of a steam distillate of coffee afforded no reliable index of coffee quality but thought that some indication of the loss of aroma during storage or manufacture was obtained. Natarajan *(5)* and his co-workers have reported the use of this method (Wurtziger) in order to evaluate Indian coffees and to detect substitution in coffee. They used permanganate in alkaline solution as the oxidizing agent in place of the dichromate of Hogl and Wurtziger. They did not use a direct steam distillation but instead used a sample of the coffee itself, together with a glass jar which held the sample, and permitted direct titration without loss of volatile materials during the test. The coffee was subjected to a high temperature (105° C.) for a long period of time (2 hours) in order to drive the volatiles into the alkaline permanganate. The number of ml. of 0.1 *N* permanganate necessary for the oxidation of the aromatic substances from one gram of coffee was taken as the oxidation value. The results were comparable to those reported by Hogl and Wurtziger.

A serious objection to these methods is that they measure the aroma of coffee that has undergone very unusual and very drastic treatment. Steam distillation might cause some chemical change of its own by virtue of the conditions set up. Heating the coffee for two hours at 105° C. is likely to cause changes by pyrolysis and decomposition of some of the more characteristic flavor components. It does not seem that either the method of Hogl or of Natarajan serves as a true or useful measure of the aromatic value of coffee. Some means of measuring this quality, which does not involve such drastic or unusual treatment of the coffee, appears desirable.

Lang and Farber *(4)* suggested the possibility of measuring the volatile reducing substances in coffee as a means of estimating its quality. The method used was one in which purified air was passed over samples of ground roasted coffee and then bubbled through alkaline permanganate. The degree of reduction of the permanganate was a measure of the volatile reducing substances present in the coffee.

Karel, Proctor, and Cornell *(3)* have recently suggested that Farber's technique could be used as a quantitative measure of the volatile reducing substances (VRS), but that it would be necessary to limit that reduction to 20% of the theoretical reducibility of the permanganate.

With these precautions in mind, work was carried out to determine whether a method utilizing the principles described by Lang and Farber and by Karel, Proctor, and Cornell could be applied to measurement of the volatile value of coffee and to determine the influence of high temperature holding on the VRS in coffee brew.

EXPERIMENTAL

Brew. Standard coffee brew was prepared according to the recommendations of the Coffee Brewing Institute (85 g. of drip ground coffee plus 1330 ml. of redistilled water).

Holding temperatures and times. Standard experimental conditions were selected as follows: Holding temperatures of 63° C., 73° C., 83° C., and 93° C., and holding times of 0, 1, 3, 6, and 24 hours.

Blend. The blend composition of the coffee studied was 30% Santos (Brazil), 35% Armenia (Colombia), and 35% Mara (Venezuela).

Organoleptic evaluation. Data were taken from the study by Segall and Proctor *(6)* which was carried out concurrently with the work on volatile reducing substances (VRS).

Measurement of VRS. Volatile reducing substances in the standard coffee brew were measured by a modification of the technique of Lang, Farber, Beck, and Yerman *(4)*, incorporating the suggestions of Karel, Proctor and Cornell *(3)* regarding the limitation of reduction to 20% of the theoretical value for the available potassium permanganate. The standard coffee brew (12.5 ml.) was mixed with redistilled water (37.5 ml.) and placed in a Lang-Farber aeration flask. Pre-

[a] Contribution No. 364 from the Department of Food Technology, M.I.T.

[b] Director of Research, Kwik Kafe Coffee Processors of America, Inc., Hatboro, Pennsylvania.

TABLE 2

Recovery of volatile reducing substances (VRS[1]) from standard coffee brew

Time of holding (hours)	Temperature of holding (° C.)							
	63		73		83		93	
	VRS/100 ml. std. brew	% VRS at "O"	VRS/100 ml. std. brew	% VRS at "O"	VRS/100 ml. std. brew	% VRS at "O"	VRS/100 ml. std. brew	% VRS at "O"
0	250	.....	250	.....	250	.....	250	.....
1	195.0	78.0	193.5	77.4	159.0	61.6	118.0	47.2
3	121.5	48.6	119.5	47.8	137.5	55.0	82.5	33.0
6	71.9	28.8	69.7	27.9	42.0	16.8	42.0	16.8
24	46.5	18.6	43.2	17.3	32.4	13.0	11.3	4.5

[1] VRS = Volatile Reducing Substances = Microequivalents of Reduction.

purified nitrogen was used as a sweeping agent and the volatiles collected and measured in 0.02 *N* alkaline permanganate.

RESULTS AND DISCUSSION

The sample size of coffee brew used for analysis was carefully selected so that it would effect less than the 20% maximum potassium permanganate reduction suggested by Karel, Proctor and Cornell *(3)* in their studies of the VRS technique. The VRS content (micro-equivalents of reduction of a standard 0.02 *N* $KMnO_4$ solution expressed as VRS) of the freshly prepared standard coffee brew was found to be 250 μ-equivalents per 100 ml.

The VRS content decreased directly as the length of holding and the temperature of holding increased. The VRS values decreased by approximately 22% when held for 1 hour at 63° C. and 73° C., while decreasing from 36% to 53% at 83° C. and 93° C., respectively. The decrease in the VRS value of the coffee brew was steady, and at the end of 24 hours was about 82% to 87% for holding temperatures of 63° C., 73° C., and 83° C., and approximately 95% at 93° C. (Table 2).

The loss of flavor in the coffee brew *(6)* was noted by the decrease in the organoleptic ratings given by the taste panel (Table 1). At each of the given holding temperatures, the decrease in the VRS content of the coffee brew seemed to parallel the decrease noted in the organoleptic acceptance ratings. (Tables 1 and 2).

TABLE 1

Organoleptic analysis of standard coffee brew[1]

Time of holding (hours)	Mean hedonic rating at temperature of holding (° C.)			
	63	73	83	93
0	7.36 ± .16	7.36 ± .16	7.36 ± .16	7.36 ± .16
1	6.17 ± .35	6.12 ± .40	6.34 ± .40	6.84 ± .26
3	6.23 ± .28	5.72 ± .51	6.00 ± .52	4.89 ± .36
6	4.77 ± .37	5.00 ± .47	3.84 ± .36	3.98 ± .39
24	2.78 ± .29	2.39 ± .33	2.72 ± .39	2.00 ± .23

[1] Segall and Proctor, 1957 *(6)*.

The VRS determination clearly shows the effect of the severe heat treatment afforded to samples at 93° C. It also shows the degree of effect at lower temperatures, and at various times of holding at elevated temperatures. The method shows that it is possible to evaluate the volatile value of a coffee brew objectively.

Additional studies should be carried out to determine the full potentialities of the method as a means by which cup testing of coffee for the evaluation of flavor decrease may be supplemented, partially at least, by an objective evaluation. It is believed that this method is superior to those previously used in that it offers a means of evaluating a coffee in the same state in which it is normally consumed. The determination is also carried out at room temperature, and not at some high temperature which may well cause changes in the coffee flavor.

SUMMARY AND CONCLUSIONS

The volatile reducing substances content of standard coffee brew was determined by the method of Lang, Farber, Beck, and Yerman *(4)*. It was found that volatile reducing substances were sensitive to the length of time coffee brew was held. The data showed that this class of compounds decreased rapidly on holding, even at the lower holding temperatures. It is concluded that the volatile reducing substances content is an indication of the age and treatment afforded a coffee brew. It should be possible to utilize this factor in establishing standards for coffee brew, and as an objective means for supplementing organoleptic evaluation.

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Relationships of Apple Grade and Size to Apple Value in Processing of Apple Slices

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DURING THE 1957–1958 apple processing season, the departments of Horticulture and Agricultural Economics, Virginia Agricultural Experiment Station, continued their cooperative work on apple processing, completing a study of the time requirements for preparation of apples for processing of apple slices and the recovery of apple fractions in the course of preparation of the slices. Studies of the same factors for apple sauce and preliminary work on apple slices were made in preceding years. These earlier studies will be useful for comparative purposes *(2, 3)*. Descriptive results of this study, comparable with that for apple sauce *(3)*, are reported in another paper *(4)*.

EXPERIMENTAL

Four plants processing apple slices were studied during the 1957–1958 season. York Imperial variety apples were used in the study. They came either from growers regularly supplying these processors or from the processors' cold storage. Apples were presized mechanically into 4 size classes: (a) less than 2¼ inches in diameter, (b) 2¼ to 2½ inches in diameter, (c) 2½ to 2¾ inches in diameter, and (d) 2¾ inches and larger in diameter. A portion of each of the 3 larger size groups were separated by Federal-State fresh fruit inspectors into U. S. No. 1, U. S. No. 2, and culls on the basis of United States Department of Agriculture standards for processing apples *(5)*. Only the U. S. No. 1 and U. S. No. 2 grade apples were used for further study. The remaining apples in each size group were separated into 4 quality groups by the same inspectors on the basis of standards proposed by the Virginia State Horticultural Society's Industry Advisory Committee. The proposed standards differ from the United States standards only in the tolerance allowed for trim waste in addition to normal preparation waste. The United States grades and experimental grades used in this study have maximum trim waste tolerances as follows:

U. S. No. 1	5.0%
U. S. No. 2	25.0%
Grade A	3.125%
Grade B	6.25%
Grade C	12.50%

The above 5 grades, in each of the 3 size classes, were run separately through a portion of the regular processing line in each plant under commercial conditions. Each experimental lot required approximately 45 minutes preparation time. Each lot was preweighed and peeled and cored on semi-automatic peelers, which counted the apples as they were peeled. Plant personnel trimmed and sliced the apples under production line conditions. Trimming times and yields of peels and cores, broken slices, trim waste, and apple slices were determined for each lot. In addition, a 15-pound, representative sample of trim waste was collected and sorted into 2 batches. One batch was trim waste which resulted from the removal of peel fragments missed by the automatic peeler (peel trim). The other batch resulted from the removal of all types of defects (defect trim). The peel and defect trim data from these samples were used to estimate the total defect and peel trim in the different experimental lots.

Data were analyzed by a covariance technique known as differential contrast *(1)*.

RESULTS AND DISCUSSION

Estimating equations were derived from these data to determine (a) peels and cores in relationship to the plant worked in and size class of apples utilized; (b) broken slices recovered in relationship to the plant worked in, size class of apples uttilized, and total trim waste experienced; (c) recovery of prepared apple slices in relationship to the plant worked in, peel and core waste experienced, broken slices obtained, and total trim found; (d) time requirements for trimming 100 pounds of raw fruit in relationship to the plant work in, size class of apples utilized, and per cent defect trim removed; and (e) total and defect trim in relationship to plant worked in, size of apples utilized, and grade of apples used. In addition, in instances where the size of apples was a factor, the average linear effect on the dependent factor of an increase or decrease in the number of apples per 100 pounds within size classes was determined. This effect is used to adjust the values of a given plant to the value of the average of all plants or to the value of another plant for comparative purposes.

The 6 estimating equations are shown below. Differential regression values, standard errors and per cent determination coefficients for these equations are shown in detail in Tables 1 to 6 in the Appendix.

Estimating Equations

1. Per cent peel and core waste = $25.60 + P_i + S_i \pm .014 d_{\bar{S}_i}$

   Where: P_i = Plant (P_A is included in the intercept)
   S_i = Size Class (S_i is included in the intercept)
   $d_{\bar{S}_i}$ = Difference in number of apples per 100 pounds from the average number of apples per 100 pounds in the size class.

2. Per cent broken slices = $7.32 + P_i + S_i + .0508X_1 \pm d_{\bar{S}_i}$

   Where: P_i = Plant (P_A is included in the intercept)
   S_i = Size Class (S_i is included in the intercept)
   X_1 = Per cent total trim waste
   $d_{\bar{S}_i}$ = Difference in number of apples per 100 pounds from the average number of apples per 100 pounds in the size class.

3. Recovery of prepared slices = $94.26 + P_i - .9715X_1 - .8929X_2 - .9202X_3$

   Where: P_i = Plant (P_A is included in the intercept)
   X_1 = Per cent peel and core waste
   X_2 = Per cent total trim waste
   X_3 = Per cent broken slices

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4. Trimming time per 100 pounds = $35.12 + P_i + S_i + 1.6835X_1 \pm .0648d_{\bar{S}_i}$

Where: P_i = Plant (P_A is included in the intercept)
S_i = Size Class (S_1 is included in the intercept)
X_1 = Per cent defect trim waste
$d_{\bar{S}_i}$ = Difference in number of apples per 100 pounds from the average number of apples per 100 pounds in the size class.

5. Total trim waste = $4.30 + P_i + S_i + G_i \pm .0036d_{\bar{S}_i}$

Where: P_i = Plant (P_A is included in the intercept)
S_i = Size Class (S_1 is included in the intercept)
G_i = Grade Class (G_1 is included in the intercept)
$d_{\bar{S}_i}$ = Difference in number of apples per 100 pounds from the average number of apples per 100 pounds in the size class.

6. Defect trim waste = $.89 + P_i + S_i + G_i \pm .0058d_{\bar{S}_i}$

Where: P_i = Plant (P_A is included in the intercept)
S_i = Size Class (S_1 is included in the intercept)
G_i = Grade Class (G_1 is included in the intercept)
$d_{\bar{S}_i}$ = Difference in number of apples per 100 pounds from the average number of apples per 100 pounds in the size class.

The foregoing 6 equations are used to estimate yield of apple fractions and trimming time requirements for apples of varying size and quality. These values may be estimated for the average of the 4 apple slices processing plants included in the study, the individual plants, or even for plants not included in the study. For the latter use some calculations from plant data are required.

Deriving intercept values for outside plants. All that is needed to adapt these estimating equations to processing plants not included in the present study is plant average figures for yield of apple fractions and trimming time requirements. These data are usually available from plant records or may be closely approximated from simple sampling studies in the plant. The plant data would be used in deriving the intercept values for the given plant.

Derivation of the intercept term in equation 1 would require the average per cent peel and core waste and the average size of the fruit utilized in the plant expressed as the number of apples per 100 pounds of fresh fruit. If the average peel and core waste in a given plant was 22.00% and there were 375 apples per 100 pounds on the average in the apples used in the plant, the new intercept value, which would include the values for small sized fruit, would be derived as follows:

$$\hat{a}'_i = \bar{Y} + b\,d_{\bar{S}_i}\,(\bar{S}_1 - \bar{S}) = 22.00 + .014\,(511 - 375 = 23.90\%$$

Where:

$\bar{Y}$ = The average peels and cores in the plant
$b\,d_{\bar{S}_i}$ = The regression for size, from the average for the size class being estimated, on peels and cores
$\bar{S}_1$ = The average number of apples per 100 pounds in size class 1 for the plants in the study
$\bar{S}$ = The average number of apples per 100 pounds in the plants being estimated

This new $\hat{a}'$ value would be substituted into equation 1 for the intercept plus plant value thus:

Equation 1: $\hat{Y} = (25.60 + P_i) + S_i \pm .014\,d_{\bar{S}_i}$

Revised: $\hat{Y} = 23.90 + S_i \pm .014\,d_{\bar{S}_i}$

The derivation of the new intercept term for equation 2 used for estimating broken slices would require the average per cent broken slices and average per cent total trim waste in the plant in addition to the average number of apples given above. The new intercept for estimating broken slices, assuming 9.0% average broken slices and 7.0% total trim waste for the plant, would be derived as follows:

$$\hat{a}'_i = \bar{Y} + b\,d_{\bar{S}_i}\,(\bar{S}_1 - \bar{S}) - b\,X_1\,(\bar{X}_1 - \bar{X}) = 9.00 + .0133\,(511 - 375) - .0508\,(5.68 - 7.00 = 10.87\%$$

Where:

$\bar{Y}$ = The average per cent broken slices in the plant
$b\,d_{\bar{S}_i}$ = The regression for size, from the average for the size class being estimated, on broken slices
$\bar{S}_1$ = The average number of apples per 100 pounds in size class 1 for the plants in the study
$\bar{S}$ = The average number of apples per 100 pounds in the plant
$b\,X_1$ = The regression of per cent total trim on broken slices
$\bar{X}_1$ = The average total trim in the plants included in the study
$\bar{X}$ = The average total trim in the plant

The new intercept value would be substituted into equation 2 in place of $(7.32 + P_i)$ as shown in equation 1.

The derivation of the new intercept for equation 3 for estimating yield of prepared apple slices would require, in addition to the information already indicated, the average yield of prepared apple slices for the plant. Assuming the average yield of prepared slices to be 64.00% the new intercept would be derived as follows:

$$\hat{a}_i' = \bar{Y} + b\,X_1\,(\hat{X}_1) + b\,X_2\,(\hat{X}_2) + b\,X_3\,(\hat{X}_3) = 64.00 + .9715\,(23.90) + .8929\,(7.00) + .9202\,(10.87) = 103.46\%$$

Where:

$\bar{Y}$ = Average yield of prepared apple slices for the plant
$b\,X_1$ = The regression of peels and cores on yield of slices found in the study
$\hat{X}_1$ = Estimated yield of peels and cores for the plant
$b\,X_2$ = Regression of total trim on yield of slices found in the study

$\hat{X}_2$ = Estimated yield of total trim for the plant

$b\,X_3$ = Regression of broken slices on yield of slices found in the study

$\hat{X}_3$ = Estimated yield of broken slices for the plant

The new intercept term would be substituted into equation 3 in place of $(94.26 + P_1)$ as in equation 1.

The derivation of the new intercept value for equation 4 used in estimating trimming time would require an estimate of average time required to trim 100 pounds of apples in the plant and an estimate of the per cent defect trim waste for the plant. Assuming that the average trimming time for the plant is 34.00 minutes per 100 pounds and that per cent defect trim is 4.46% in the plant, the new intercept would be derived as follows:

$$\hat{a}_1' = \overline{Y} + b d_{\overline{S}_1} (\overline{S}_1 - \overline{S}) + b\,X_1 (\overline{X}_1 - \overline{X}) = 34.00 + .0648\ (511 - 375) + 1.6835\ (4.46 - 3.14) = 45.03 \text{ minutes}$$

Where:

$\overline{Y}$ = The average trimming time per 100 pounds of fresh apples in the plant

$b\,d_{\overline{S}_1}$ = The regression for size, from the average apples per 100 pounds in the size class being estimated, on trimming time

$\overline{S}_1$ = Apples per 100 pounds in size class 1 in the plants in the study

$\overline{S}$ = Apples per 100 pounds in the plant

$b\,X_1$ = The regression of defect trim on trimming time

$\overline{X}_1$ = Per cent defect trim in the plant

$\overline{X}$ = Average defect trim for the U. S. No. 1 grade apples in the 4 plants in the study

The new intercept term would be substituted in equation 4 in place of $(35.12 + P_1)$.

The new intercepts for equations 5 and 6 used in estimating total trim and defect trim for apples of different size and grade would be somewhat more difficult to determine. The total trim and defect trim figures determined from plant data 7.00 and 4.46% respectively would be adjusted to the S_1 size classification as follows:

Total trim $(S_1) = 7.00 + .0036\ (511 - 375 = 7.50\%$

Defect trim $(S_1) = 4.46 + .0058\ (511 - 375) = 5.25\%$

Next the proportion of the different grade classes run through the plant would need to be determined. For instance if 65% of the apples utilized in the plant were U. S. No. 1 quality, 30% were U. S. No. 2 and 5% were culls; the approximate per cent total trim for U. S. No. 1 apples in the plant could be determined as follows:

$$100\ (7.50) = 65X + 30\ (X + 4.68) + 5\ [X + 2\ (4.68)]^b$$

$$750 = 65X + 30X + 140.4 + 5X + 46.8$$

$$100X = 750 - 140.4 - 46.8 = 562.8$$

$$X = 5.63\% \text{ total trim in } G_1 \text{ class}$$

A similar procedure would estimate defect trim waste at 3.51% as follows:

$$100\ (525) = 65X + 30\ (X + 3.87) + 5\ [X + 3\ (3.87)]^c$$

$$525 = 65X + 30X + 116.1 + 5X + 58.05$$

$$100X = 350.85$$

$$X = 3.51\%$$

Average yields of apple fractions and time requirements. Yields of apple fractions and trimming time requirements for apple slices processing, based on the average of the 4 plants included in the study are shown in Tables 1, 2, 3 and 4. The yields and times were adjusted to the same number of apples per 100 pounds in the 3 size classes as were found in the study of applesauce processing *(2)* and as in the applesauce study maximum trim waste allowed in the grade was asumed as total trim waste.

Variation in size of apples was found to be the only factor related to peel and core waste for apple slices while for applesauce both the size of apples and trim waste were factors. As apples for slices are usually peeled more heavily than apples for sauce, this development is easily explained. Surface defects have little or no effect on a peeling knife that is running well below the surface. Therefore, for slices, estimated peel and core waste is shown only for the 3 size classes and would be the same for all grades within a size class (Table 1).

TABLE 1

Estimated per cent peel and core waste of apples of different size, processed for apple slices, average in four plants 1957–58 season

Size class	Peel and core waste
	pounds per 100 lb.
2¼ to 2½ inches [1]	28.21
2½ to 2¾ inches [1]	25.71
2¾ inches and larger [1]	24.79

[1] Five hundred and twenty-six, 418, 278 apples per hundred pounds, respectively, for the small, medium and large size classes (diameter in inches).

Broken slices were not a factor, of course, in the applesauce study as apples for applesauce are not sliced. Both smaller size and per cent trim waste regardless of type of trim result in weaker slices providing the same slicing knives are used in slicing. The amount of recovered broken slices in the different grade and size classes of apples are shown in Table 2.

The recovery of prepared slices was affected by the per cent broken slices in addition to the peel and core waste and total trim waste found to affect applesauce recovery. The results for the average of the 4 plants for apple slices are shown in Table 3.

[b] Assuming that culls would have twice the trim waste of U. S. No. 2 grade.

[c] Assuming that additional defect trim in culls over U. S. No. 2 would be 3 times as great as in U. S. No. 2 apples.

TABLE 2

Estimated per cent broken slices by size and grade class, average in four plants, 1957–58 season, maximum trim waste for grade assumed as total trim waste

Size class	Grade				
Diameter in inches	U. S.		Experimental		
	1	2	A	B	C
	pounds per 100 lbs.		*pounds per 100 lb.*		
2¼ to 2½	8.26	9.28	8.17	8.33	8.65
2½ to 2¾	6.76	7.78	6.67	6.83	7.15
2¾ and larger	4.96	5.98	4.87	5.03	5.35

TABLE 3

Estimated recovery of prepared apple slices by size and grade class, average in four plants, 1957–58 season, maximum trim waste in grade assumed as total trim waste

Size class	Grade				
Diameter in inches	U. S.		Experimental		
	1	2	A	B	C
2¼ to 2½	55.73	36.93	57.48	54.54	48.67
2½ to 2¾	59.54	40.74	61.29	58.36	52.48
2¾ and larger	62.10	43.30	63.85	60.91	55.04

Variations in trimming time of apples for production of apple slices were affected by plant differences, variation in size, and variation in per cent defect trim. Because peel trim remained relatively constant among plants, grades, and sizes of apples, it was not an important factor in affecting variation in trimming time with slices as it was with applesauce. Trimming times for the different size and grade classes in the 4 plants are shown in Table 4.

TABLE 4

Estimated trimming time requirements for apples for apple slices, average in four plants, 1957–58 season, maximum trim waste allowed in the grade assumed as total trim waste

Size class	Grade				
Diameter in inches	U. S.		Experimental		
	1	2	A	B	C
2¼ to 2½	36.16	57.71	34.14	37.50	44.24
2½ to 2¾	29.76	51.31	27.74	31.10	37.84
2¾ and larger	20.09	41.64	18.07	21.43	28.17

Determining the relative value of different grades and sizes of apples for processing apple slices. The information in Tables 1 through 4 may be used for determination of the relative value of apples of different grades and sizes for processing of apple slices. Methods used for the applesauce study *(2)* would be used, substituting apple slices data for applesauce data, and modifying the assumptions of plant cost relationships and value of waste product used in the applesauce study to provide for apple slices. For instance, it is likely that labor costs are a somewhat greater percentage of total costs in a slice processing plant as trimming is somewhat more exacting and another operation is required in slices preparation. The broken slices fraction in processing slices is more valuable than peel, core, and trim waste, especially if the plant is canning applesauce simultaneously.

As average plant cost relationships are not available and as each plant would likely arrive at a different basis of cost relationships in determining relative prices in their own plant, little would be gained by again making assumptions as to plant cost relationships in this paper. It is likely that the differentials for grade and size shown previously *(2)* would be in the same direction and somewhat greater for slices than for applesauce. The adoption of the experimental grades would be even more beneficial to the high quality large apple size growers and to the processors if the apples were purchased on the basis of these or other closer tolerance grades for apples for processing in processing apple slices than they were for processing applesauce.

The need for a revision of sampling methods. Various sampling procedures are now used for determining grade and size of growers' fruit. Most commonly, some predetermined number of field containers are selected at random from the grower's load during the unloading process. Containers selected are designated by the Federal-State inspector or by persons working under his supervision. Samples are composited and the apples in the sample are separated into the various grade and size classifications according to the basis of payment used by the processor. For instance, an apple judged by the inspector to have less than five per cent waste in addition to normal peel and core waste would be placed in grade U. S. No. 1, and furthermore would be subclassified according to its diameter. Where a plant is buying on the basis of 2½ inches in diameter and up, all apples not passing through the 2½ inch sizing ring would be placed in the highest classification bin, those that passed through the 2½ inch ring but would not pass through the 2¼ inch ring would be placed in a lower class bin, and the remainder would be placed in ciders. After the entire sample is inspected, the various portions are weighed and related to the original weight of the sample. The resulting percentage figures are then applied to the net weight of the load that was sampled to arrive at the basis for payment.

Assuming for the moment that the sample drawn is perfectly representative of the load, the present system is still inadequate to serve as a basis of payment. Actual size of apples vary greatly even within a ¼ inch in diameter size range and according to research results these differences are sufficient to materially affect costs of processing. The variation that in this study was found to occur within the small size classification of apples, would account for 1.89% variation in yield of apple slices. There was a maximum spread of 65 apples per 100 pounds among the 2¼ to 2½ inch in diameter classes. Other studies showed even greater variation in this size class than the lots in this study showed. In the medium size classification, yield would vary by as much as 2.92% due to size with a maximum spread between lots of 103 apples per 100 pounds. For the largest apple size class the variation in yield due to size variation within the class was as much as 1.54% with a maximum spread of 58 apples per 100 pounds in the class. Other studies have shown

that the maximum spread in this class could be much larger than that which occurred in this study. Trimming time requirements could have varied by 4.21, 6.67, and 3.76 man-minutes per 100 pounds respectively due to the variation that in this study occurred in the number of apples per 100 pounds in the size classes small, medium, and large respectively.

It is apparent that if we are to develop a finer measuring stick in purchasing processing apples, some modification of size measurement is necessary. Counting the fruit in the sample to arrive at the number of apples per 100 pounds of apples would provide this needed refinement. Some arbitrary apple count per 100 pounds could be selected as a base in buying apples with premiums and discounts set up for deviations from this base.

Samples obtained by present methods may not accurately reflect the quality of the load of fruit being sampled. In compositing the samples it is not possible to determine the variation that occurs between samples. A means should be developed for making a continuous random sample of the entire load during the unloading process, at least for those loads that are run directly into the processing plant bins. For loads that are taken into storage for later use this method might not be useable and an adequate sample of the present type might be the only practical solution. A continuous random sampling procedure would not only provide a more representative sample, but would likely require a smaller sample; thus increasing the efficiency of the inspection job. In some plants a system of intermittent diversion of apples from the elevators during the unloading period by manual control of diversion gates extending onto the conveyor belt has been used. This is an improvement over the more common method of sampling, but likely this method could be improved upon by development of a mechanical sampler that would take a large number of smaller samplings from the conveyor. Work along this line is greatly needed and is long overdue.

SUMMARY AND CONCLUSIONS

A correlative study with that done for applesauce processing was completed for processing apple slices in the 1957–58 processing season. Four apple slices processing plants cooperated with the Virginia Agricultural Experiment Station in this study. The study was designed to determine the effects on recovery of apple fractions and preparation time requirements of grade and size of fruit in processing of apple slices under commercial conditions. The present United States grades No. 1 and No. 2 and three experimental grades proposed by the Virginia Horticultural Society's Industry Advisory Committee in each of three size classes were studied.

Estimating equations were derived from the experimental data by a statistical procedure known as differential contrast to estimate the recovery of peels and cores, total trim, defect trim, broken slices, and prepared apple slices when apples of the different grades and sizes were utilized. The trimming time requirements for the different grades and sizes were also estimated.

As processing plants other than those included in the study might wish to use the estimating equations that were developed, a methodology for deriving the intercept values for plants not included in the study was shown, using data that is either obtainable from usual plant records or that could be developed quite readily by plant management.

Average yields of apple fractions and trimming time requirements for the four plants included in the study are shown. These values may be used to determine the relative value of the different grades and sizes of apples by utilizing the methods shown in the study of applesauce processing *(2)*.

The refining of apple grades indicated as being to the advantage of high quality large apple size producers points also to a need for a refinement of our apple size classifications. The variation in size that occurs within a ¼ inch range in size is sufficient to materially affect both recovery of prepared product and preparation time requirements. A measurement of size on the basis of the number of apples per 100 pounds of fresh fruit is suggested as an alternative to the present diameter size classification. Also the problem of more accurately and efficiently obtaining a measure of the quality of apples delivered to the plants is of great importance to both growers and processors. A continuous random sampling procedure is suggested as worthy of consideration and research.

APPENDIX

TABLE 1

Regression coefficients, standard errors, and the per cent determination in estimating per cent peels and cores waste, 1957–58 apple slices study

Item	Constant	Coefficient	Standard error
Intercept [1]	a'	25.60[2]	0.51
Plant B	P_B	1.32[2]	0.58
Plant C	P_C	0.52[4]	0.58
Plant D	P_D	7.76[2]	0.58
Size 2 ½ to 2 ¾ inches	S_2	−2.82[2]	0.53
Size 2 ¾ inches and larger	S_3	−3.35[2]	0.51
Differential for size	$d-_{S_1}$	0.0140	
Per cent determination	R^2	0.8254[2]	

[1] Includes the values for P_A and S_1 (2 ¼–2 ½ inches).
[2] Significant at the 1% level with 59 degrees of freedom.
[3] Significant at the 5% level with 59 degrees of freedom.
[4] Not significant at the 1 or 5% level with 59 degrees of freedom.

TABLE 2

Regression coefficients, standard errors, and per cent determination in estimating per cent broken slices, 1957–58 season, apple slices study

Item	Constant	Coefficient	Standard error
Intercept [1]	a'	7.32[2]	0.31
Plant B	P_B	−1.80[2]	0.27
Plant C	P_C	2.19[2]	0.27
Plant D	P_D	1.57[2]	0.27
Size 2 ½ to 2 ¾ inches	S_2	−1.81[2]	0.23
Size 2 ¾ inches and larger	S_3	−3.23[2]	0.24
Per cent total trim	X_1	0.0508[2]	0.0306
Differential for size	$d-_{S_1}$	0.0033	
Per cent determination	R^2	0.8936[2]	

[1] Includes the values for P_A and S_1 (2 ¼–2 ½ inches).
[2] Significant at the 1% level with 58 degrees of freedom.
[3] Not significant at the 1 or 5% levels with 58 degrees of freedom.

TABLE 3

Regression coefficients, standard errors and the per cent determination in estimating yield of prepared slices, 1957–58 season, apple slices study

Item	Constant	Coefficient	Standard error
Intercept [1]	a'	94.26[2]	2.53
Plant B	P_B	2.95[3]	0.73
Plant C	P_C	.54[3]	0.72
Plant D	P_D	2.25[4]	1.02
Peel and core (per cent)	X_1	0.9715[2]	0.134
Total trim (per cent)	X_2	0.8929[2]	0.0676
Broken slices (per cent)	X_3	0.9202[2]	0.1916
Per cent determination	R^2	0.9346[2]	

[1] Includes values for P_A.
[2] Significant at the 1% level with 58 degrees of freedom.
[3] Not significant at the 1 or 5% levels with 58 degrees of freedom.
[4] Significant at the 5% level with 58 degrees of freedom.

TABLE 4

Regression coefficients, standard errors and per cent determination in estimating trimming time per hundred pounds of apples, 1957–58 season, apple slices study

Item	Constant	Coefficient	Standard error
Intercept [1]	a'	35.12[2]	1.25
Plant B	P_B	−14.47[2]	1.28
Plant C	P_C	− 3.59[2]	1.38
Plant D	P_D	− 3.21[2]	1.27
Size 2 ½ to 2 ¾ inches	S_2	− 7.89[2]	1.17
Size 2 ¾ inches and larger	S_3	−15.75[2]	1.12
Per cent defect trim	X_1	1.6835[2]	0.1541
Differential for size	$d-_{S_1}$	0.0648	
Per cent determination	R^2	0.8887[2]	

[1] Includes the values for P_A and S_1 (2 ¼–2 ½ inches).
[2] Significant at the 1% level with 58 degrees of freedom.
[3] Significant at the 5% level with 58 degrees of freedom.

TABLE 5

Regression coefficients, standard errors, and the per cent determination in estimating total trim waste, 1957–58 season, apple slices study

Item	Constant	Coefficient	Standard error
Intercept [1]	a'	4.30[2]	0.85
Plant B	P_B	1.81[2]	0.85
Plant C	P_C	2.77[2]	0.85
Plant D	P_D	0.65[4]	0.85
Size 2 ½ to 2 ¾ inches	S_2	−0.15[4]	0.69
Size 2 ¾ inches and larger	S_3	−0.89[4]	0.66
Grade 2	G_2	4.68[2]	0.75
Grade A	G_A	−0.52[4]	0.75
Grade B	G_B	2.90[2]	0.75
Grade C	G_C	5.01[2]	0.85
Differential for size	$d-_{S_1}$	0.0036	
Per cent determination	R^2	0.6265[2]	

[1] Includes the values for P_A and S_1 (2 ¼–2 ½ inches) and G_1, (U. S. No. 1).
[2] Significant at the 1% level with 55 degrees of freedom.
[3] Significant at the 5% level with 55 degrees of freedom.
[4] Not significant at the 1 or 5% level with 55 degrees of freedom.

TABLE 6

Regression coefficients, standard errors, and per cent determination in estimating defect trim, 1957–58, apple slices study

Item	Constant	Coefficient	Standard error
Intercept [1]	a'	0.89[2]	0.94
Plant B	P_B	1.46[3]	0.83
Plant C	P_C	3.75[3]	0.83
Plant D	P_D	0.61[3]	0.83
Size 2 ½ to 2 ¾ inches	S_2	0.38[2]	0.76
Size 2 ¾ inches and larger	S_3	1.41[2]	0.73
Grade 2	G_2	3.87[2]	0.94
Grade A	G_A	−0.73[2]	0.94
Grade B	G_B	2.24[4]	0.94
Grade C	G_C	4.13[2]	0.94
Differential for size	$d-_{S_1}$	0.0058	
Per cent determination	R^2	0.5445[2]	

[1] Includes the values for P_A and S_1 (2 ¼–2 ½ inches) and G_1, (U. S. No. 1).
[2] Not significant at the 1 or 5% levels with 55 degrees of freedom.
[3] Significant at the 1% level with 55 degrees of freedom.
[4] Significant at the 5% level with 55 degrees of freedom.

Acknowledgments

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Pinking of Onions During Dehydration

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(Manuscript received October 1, 1958)

DURING THE COMMERCIAL production of dehydrated onions one of the problems is the development of a pink pigment in the onion during the dehydration process. The cause is unknown, and there are no reports upon it in the literature. Many people familiar with this phenomenon believe it is caused by the growth of bacteria or fungi. There is a disease of the onion called pink root which is caused by a fungus attacking the growing bulb and causing the roots to die *(11)*. This disease, however, does not usually attack the flesh of the onion itself. Several types of food spoilage organisms will cause pink colors to develop during the processing—such as those which cause red sauerkraut or red bread *(8)*. But to ascribe the cause of pink onion to these organisms is of dubious merit since pink material can be found which has a very low bacteria count and from which no pink bacteria can be isolated. The present investigation was prompted by a desire to find the true cause of this pigmentation.

EXPERIMENTAL

The first phase of the investigation was undertaken to determine if bacteria are essential for the pigment development or whether it will form in their absence. To this end sterile purées were prepared and then checked for their ability to turn pink.

Method for determining possible role of bacteria. Several mature onions of the Southport White Globe variety were dipped into alcohol. The skin and several of the outer fleshy layers as well as the neck and root base were removed with a sterile knife. The inner part was then puréed with an equal weight of sterile water in a sterile Waring blender for 5 minutes. The bacteria counts of these preparations were well below 100 per gram. To prevent any possibility of growth from the few remaining contaminating organisms, the pH was lowered to 4.0 with 1.0 N HCl and then 1.0% sodium benzoate was added. This preparation was placed in sterile cotton stoppered flasks and incubated at 37° C. along with similar purées made without aseptic precaution and containing no germicides. At the end of 24 hours both the sterile and non-sterile preparations were very pink, the former having the deeper color. In 48 hours' incubation the controls were bleaching out and fermenting strongly while the sterile flasks were still very pink, showing no sign of bacteria growth. This experiment would indicate that not only were bacteria unessential for pigment formation, but that heavy contamination of an onion piece might cause the disappearance of some of the pigment.

If bacteria did not cause the pinking of onions, what was the causative agent? The first possibility that came to mind was the various enzyme systems, such as polyphenolase or peroxidase which will cause pink or red discoloration in many vegetative products during processing. Onions are not blanched prior to dehydration and hence these enzymes are not destroyed. Because the low temperatures used during dehydration will not cause their inactivation, they will do their work whenever the conditions enable them to do so. The probability of an enzyme reaction being involved in the pigment formation was demonstrated by the following experiment.

Method for studying influence of enzyme. Fresh onions were sliced into boiling distilled water and blanched until they were peroxidase negative; then they were puréed in a blender. This preparation did not turn pink when held in a water bath at 40° C. unless a small amount of unblanched onion juice was added to it. In a similar manner dehydrated powdered onion was reconstituted with boiling water and then held in a water bath and did not pink unless a small amount of unheated powder was added to it.

It would seem probable that the unheated onion provided the necessary enzyme system back to the purée which had had its original enzyme destroyed by heating. The amount of raw juice or powder added to the blanched preparation was not the source of the pigment as equal amounts added to distilled water and held simultaneously with that added to the blanched purées developed only a very faint color as contrasted with the deep red color of the latter. This enzyme was separated from raw onion juice by using the technique of isoelectric precipitation. The method used was similar to that employed by Seebeck and Stoll *(6)* to isolate the enzyme alliinase from garlic juice.

Several White Globe onions were now peeled and ground with an equal weight of water for 30 seconds in a blender. The juice was pressed out through cheese cloth and adjusted to pH 3.5–4.0 with diluted HCl. After standing several hours the precipitated material was filtered off and the solids were resuspended in pH 6.0 citrate-phosphate buffer. The solution was filtered and the enzyme was found in the clear filtrate.

This enzyme solution did not turn pink by itself, but when blanched onion juice was added to it, the resulting mixture turned pink if held several hours at 40° C. Heating of the enzyme solution to 90° C. for two minutes inactivated it and destroyed its ability to cause pigment formation. While the above experiments would indicate that enzymes play a strong role in the development of pinking, the embarrassing fact kept cropping up during these investigations that under some conditions the pink pigment would form in the absence of any enzyme. This was demonstrated by the following experiments.

Method for studying development of pink pigment in the absence of enzyme. Fresh onions were ground in a blender and the purée was allowed to stand a few minutes to allow the native enzyme to act. Then ethyl alcohol was added to a final concentration of 80% to precipitate the proteins. These were filtered off and the clear, non-pink filtrate was distilled off under vacuum to remove the alcohol. The preparation turned pink even though there was no enzyme present. In a similar manner dehydrated onions, if first reconstituted in cold water and then the enzyme precipitated with alcohol before any pigment develops, later turned pink in the absence of the enzyme. If hot water was used for the reconstitution, the color did not develop.

In both of these experiments with fresh and dehydrated onions it was necessary for the enzyme to act first and then the reaction would continue in its absence. At this point in the investigation it seemed probable that the pinking reaction took place in two stages. First, there was an enzyme which acted on a substrate to produce a compound which would then non-enzymatically turn pink. An effort to isolate this last compound was made so that its properties might be determined.

Fresh onions were found to be the best source of this compound. These were ground up and the juice pressed out through a cloth and then filtered clear. The resulting solution was chilled to 0° C. to slow down the reaction and various solvents were used to determine their ability to remove the color developing compound before it turned pink. It was noted during the course of the investigation that ether extraction could decrease the ability of the juice to pink. In fact, the juice could be repeatedly extracted with ether before the pigment developed—after which the aqueous residue could not develop the pigment upon standing. However, if the ether solution fraction was evaporated down and the residue added back to the aqueous residue, then the mixture would pink. Neither would produce color when held separately but would when combined.

This indicated there were at least two compounds involved in the nonenzymatic phase of pinking. One was ether soluble and the other was insoluble. Attempts to identify the active principle in the ether soluble fraction have not been successful, but the water soluble compounds have been identified.

The active ingredients in the aqueous residue of the ether extraction juice were identified by spotting the juice very heavily on a filter paper chromatograph and chromatographing it in the ascending direction using tert-butanol-formic acid-water solvent *(10)*. After the solvent had evaporated a suspension of the ether soluble fraction in pH 5.6 citrate-phosphate buffer was sprayed onto the paper and the moist paper placed in a humid incubator at 40° C. for several hours to let the non-enzymatic reaction take place. When the chromatogram was examined there was found not one spot but a whole series of pink spots. Duplicate chromatograms were sprayed with various developing agents, and it was found that the pink spots corresponded in location with that of the amino acids present on the paper. This indicated that the ether soluble fraction was reacting with the amino acids in the onion juice. That this is true can be demonstrated by the following experiment.

Method for studying role of amino acids. Onion juice made by grinding several white onions in a meat grinder and pressing through cloth was then shaken with an equal volume of ether immediately after preparation and before pink color developed. After separation the ether layer was removed and the ether distilled off. The residue was a greenish yellow paste. This was shaken with a small amount of pH 5.6 citrate-phosphate buffer and the insoluble material filtered off. The filtrate was saved as it contained the color developing compound. This solution was a slightly cloudy, almost colorless, fluid which did not turn pink by itself. However, it needed only a small amount of the amino acid glycine added to it to develop a deep lavender color after a few hours incubation at 40° C.

The ether extraction of the juice of blanched onions did not extract any compound which would develop a color when amino acids were added to it. Such a juice would, even after ether extraction, turn pink if the enzyme was added to it. This would indicate the precursor of the color developer was not ether soluble.

Figure 1 illustrates the absorption curves of the pigment extracted from dried onions which have developed pinking during dehydration. Curves are also shown of the pigment formed when the ether soluble color developer and several different amino acids were mixed.

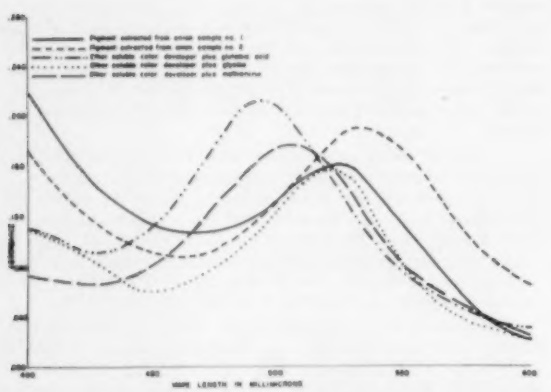


Figure 1. Absorbance of pink onion juice and isolated fractions.

The general shape of the curves is similar, but the absorption maximum varies slightly with the amino acid used. Glutamic acid shows a peak at 490 mμ, methionine at 500 mμ and glycine at 520 mμ. Two different varieties of onions were used for the two onion pigment curves. One sample peaked at 520 mμ and the other at 530 mμ.

DISCUSSION

The evidence would indicate that there is an essential enzyme step in the first phase of the pigment formation. This takes place quite rapidly after the tissues are crushed. The speed of this first step is indicated by experiments in which the juice was ether extracted and the ether layer separated from the aqueous layer within 15 minutes after crushing. When tested, this gave as good a yield of color developer as did juice which was allowed to stand an hour before extraction.

There is little in the literature in regard to the enzyme systems of onions. Peroxidase is present as can be demonstrated by the color developed by the familiar guaiacol plus hydrogen peroxide test. Catalase is present in the raw juice as indicated by the gas produced when hydrogen peroxide is added to it.

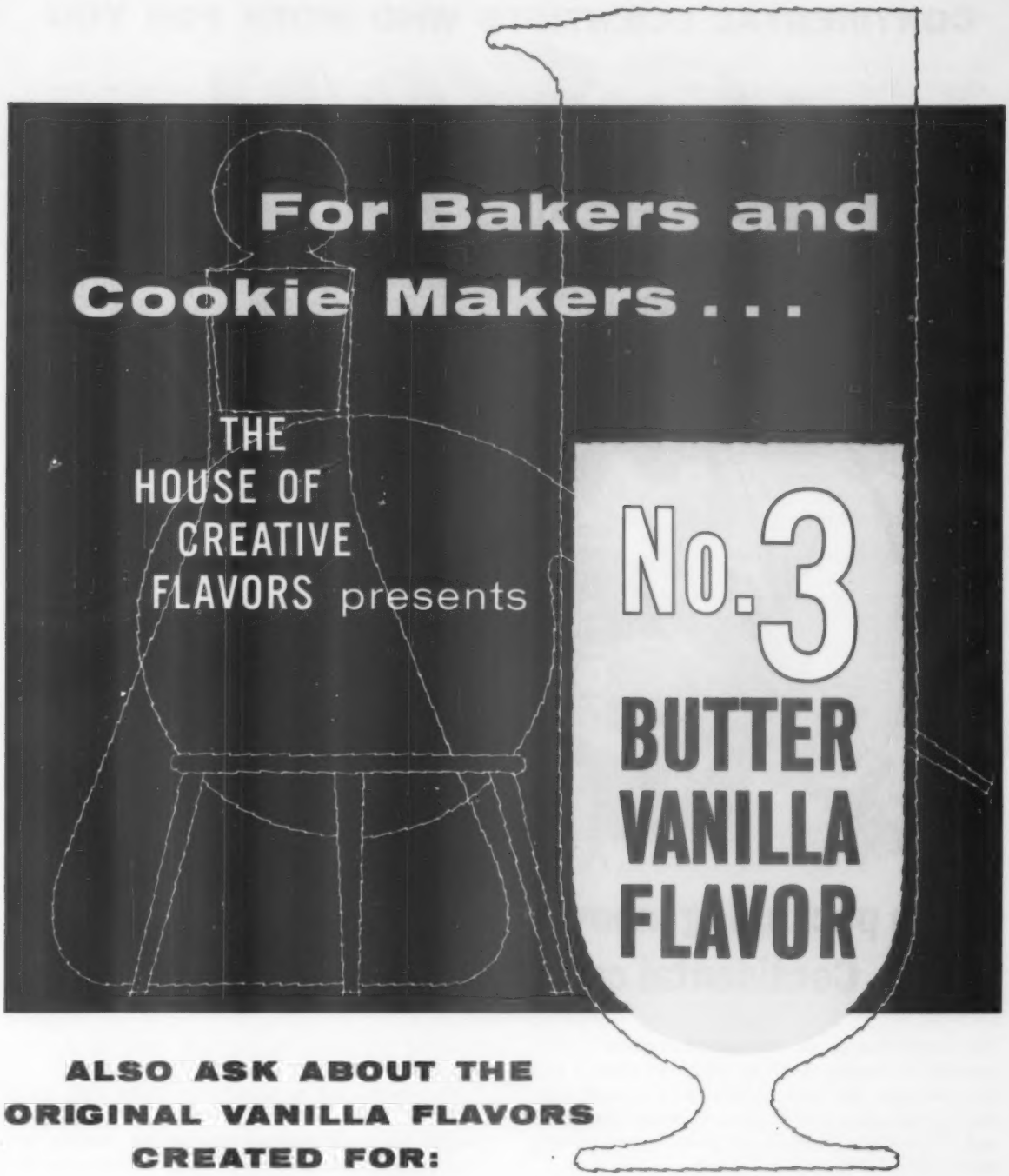
There is some similarity between the onion pigment formation and the reaction of tyrosinase on various phenolic compounds to produce pigments *(7)*. This enzyme reacts with tyrosine to produce the red pigment hallochrome. With both tyrosine and phenols, the reaction proceeds in at least two stages, the first being the actual enzyme reaction on the substrate which is then followed by a non-enzymatic condensation to produce the actual colored product.

There is an even greater similarity between pinking of onions and the reaction between phenols and amino acids which produce colored pigments *(2, 3, 9)*. This reaction also takes place in two stages. First, there is the oxidation of the polyphenol by polyphenolase to quinones which then non-enzymatically react with the amino acids present in solution to produce colored compounds.

Polyphenolase has not been found in the onions, but phenols have been found in pigmented onions but are wholly absent or if present, only in very small amounts in white onions *(1, 5)*. Checks on the ability to pink indicate that the latter are more susceptible to pinking than are yellow onions, which would lead one to question whether or not phenolic compounds are involved. The addition of guaiacol or catechol plus glycine to onion juice does not cause an increase in the amount of pinking over those aliquots which have glycine alone added.

The substrate which the enzyme acts upon to produce the non-enzymatic colorless color developer is a water soluble compound which is relatively heat stable. Long heating at the temperature of boiling water does seem to cause some decomposition. Such a preparation does not develop as much pigment as do those which have been heated only long enough to destroy peroxidase. The colorless compound resulting from the action of the enzyme on its water soluble precursor is moderately ether soluble and can be extracted with it from the juice after the raw tissues have been crushed and before the color develops. It is fairly heat stable but heating in boiling water will slowly destroy its ability to develop color with amino acids.

The non-enzymatic phase of the reaction is much slower than the enzymatic phase. Strong concentra-



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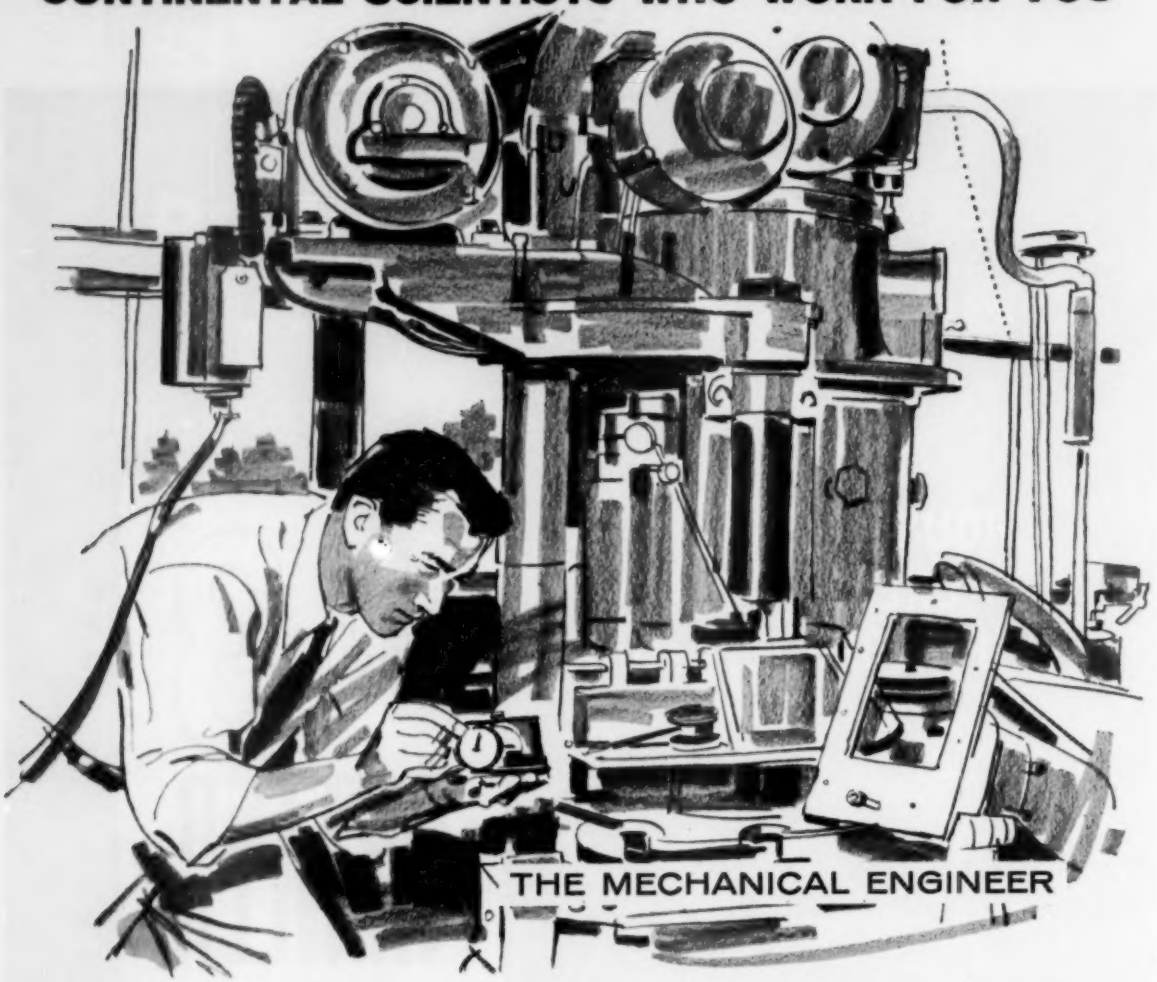

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tions of the color developer and amino acids take several hours to develop color at 40° C. The pink or lavender color will develop with a wide range of amino acids. Those tested and which developed the color are aspartic acid, glutamic acid, alpha-amino-n-butyric acid, methionine, lycine, alanine, leucine, norvaline, tyrosine, arginine, phenyl alanine, valine, serine, norleucine, isoleucine and glycine. The following would develop no color under the same conditions as the above: asparagin, histidine, glutathione, hydroxyproline, proline, tryptophane, threonine and cysteine. Distillation Products Industries amino acids were used, as received, in the above tests.

The pigment so formed is insoluble in ether, hexane, benzene and chloroform. It is soluble in water and alcohols.

Since the submission of this paper for publication another paper has been published on the pinking of onion purées *(4)*. The authors believe the pink pigment to be a new type of nitrogenous water soluble pigment differing from previously investigated plant pigments, possibly formed from Leucoanthocyanin like precursors.

SUMMARY

This investigation as to the causes for the development of a pink pigment in onions while being dehydrated has demonstrated it is not due to bacterial action. The actual course of the reaction is as follows. When the onion cells are ruptured, the native enzymes react with a substance in the juice to form a color developer. This then reacts non-enzymatically with the amino-acids in the onion to form a pink pigment.

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Changes in the Carotenoid Pigments in Preparation and Storage of Valencia Orange Juice Powder

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(Manuscript received January 23, 1959)

PROCESSED ORANGE JUICE products, such as canned single-strength juice, concentrated juice, or powder, undergo considerable deterioration on prolonged storage at room temperature. Among the changes that occur are ascorbic acid loss, darkening and development of an unpleasant off-flavor. Also, in unpublished work at this laboratory, Mylne found decreases up to 30% in total carotenoid content, measured colorimetrically, of canned Valencia orange juice after storage for 1 year at 70° F. In a study of storage changes in orange juice powder, Mylne and Seamans *(5)* noted a decrease in total carotenoids of 18% during the conditioning period (75 days at 70° F.) and a further drop of 14% during storage at 100° F. for 6 months, a total of 32%. During 100° F. storage the carotenoid content dropped rapidly initially and then leveled off. It was stated that these decreases may have been due to selective oxidation of the carotenoids most susceptible to oxidation.

The carotenoids of Valencia orange juice were investigated by the present workers *(1, 2, 3)*, using countercurrent distribution to effect an initial fractionation, followed by chromatography to isolate the individual constituents. The carotenoid mixture was shown to consist of more than 20 components, 3 of which were provitamins A. The major part of the carotenoids were xanthophyll 5,6-epoxides and their acid-catalyzed isomerization products, the xanthophyll 5,8-epoxides.[b] An investigation was later made *(4)* of the carotenoids of canned Valencia orange juice which had been stored for three years at room temperature. It was found that the xanthophyll 5,6-epoxides had entirely disappeared, while the 5,8-epoxides were present in greater proportions than in fresh juice.

A study has now been made of the changes in individual carotenoids occurring in Florida Valencia orange juice during preparation of powder, on conditioning of powder for 78 days at 77° F., and on subsequent storage for 6 months at 100° F. A comparison was also made of the composition of the carotenoid fractions from single-strength and 6-fold concentrated juices.

[a] A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[b] The 5,6- and 5,8-epoxides were referred to in earlier publications by the present authors as epoxides and furanoxides, respectively.

EXPERIMENTAL

Materials. Single-strength (very high in pulp) and 6-fold concentrated Valencia orange juices were obtained from a commercial processing plant in Florida. The 2 juices were obtained from separate batches; neither received any heat treatment. The juices were frozen, shipped to California in dry ice storage, and on arrival were stored at −10° F.

Orange juice powder was prepared by the vacuum puff-drying method as described by Strashun and Talburt *(6)*, using the concentrate to which 0.12 g. of $NaHSO_3$ and 4.2 g. of orange juice pulp (from the single-strength juice) per pound were added. The puff-dried powder, which contained 3% moisture, was passed through a 6-mesh screen and packaged in a low-humidity room in 202 x 314 cans. Each can contained 75 g. of powder, a desiccant package, and 0.8 g. of sorbitol granules (10–20 mesh) which contained 4.8% cold-pressed orange oil. Cans were sealed under 29 in. vacuum.

The powder was "conditioned" at 77° F. for 78 days in order to reduce the moisture content to 1% and then stored at 100° F. for 6 months. Lots of 4 cans each of the freshly prepared, conditioned, and stored powders were used in a study of the changes occurring in the carotenoid constituents.

Extraction of pigments. The procedure used was a modification of that previously described *(1)*. Single-strength juice after thawing was mixed with an equal volume of methanol. After standing about 30 minutes filter aid (2% by weight of the juice) was added and the mixture was filtered on a Buchner funnel precoated with filter aid. The filter cake was washed on the funnel 3 times with 50% methanol; it was then blended a short time with acetone, filtered on a sintered glass funnel, and washed with acetone until the washings were colorless. The acetone was evaporated *in vacuo*, and the residue transferred to a separatory funnel. The flask was washed once with water and then with ether (peroxide-free), ca. 1 g. of sodium chloride was added, and the mixture was extracted repeatedly with ether until the extracts were practically colorless. The combined ether extracts, with absolute alcohol added to remove water, were evaporated *in vacuo*.

The residue was dissolved in ether and transferred to a 100-ml. volumetric flask. Twenty-five ml. of a 20% solution of potassium hydroxide in methanol was added, the flask was filled with ether, and the mixture was allowed to stand in the dark overnight. The mixture was then transferred to a separatory funnel with water (9 volumes per volume of potassium hydroxide in methanol used) and ether, ca. 1 g. of NaCl was added, and the mixture extracted with ether. The combined ethereal extracts were washed 7 times with water and absolute alcohol was added. The extracts were evaporated *in vacuo*, and the residue was dissolved in the upper layer of the solvent system used in countercurrent distribution.

The concentrated juice and powders were dissolved in 2 and 4 ml. of water per gram, respectively, and the solutions worked up as described above.

Countercurrent distribution. Preparations from all 5 materials were used in 200—transfer runs in a glass Craig apparatus with the solvent system benzene, petroleum ether (Skellysolve B) and 87% methanol (1 to 1 to 1.15 by volume). The procedure used in countercurrent distribution was previously described *(1)*. At the end of the run the solution in each tube of the Craig apparatus was withdrawn with a glass syringe and transferred to a test tube. The solution left ad-

hering to the walls of the extraction tubes, transfer tubes, connecting tubes, etc., was discarded because it is practically impossible to remove it completely. This represents a loss of about 1–2% and is almost the same for each tube in the apparatus, so that it has no effect on the distribution curves (Figure 1).

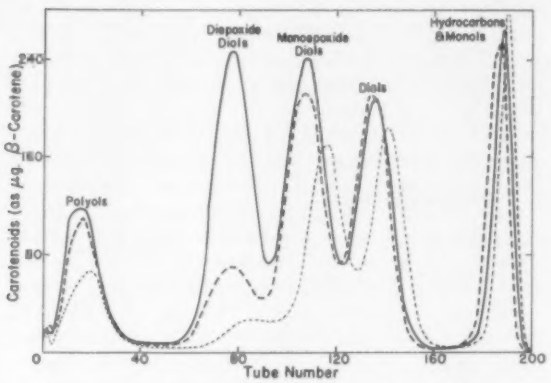

Figure 1. Countercurrent distribution of saponified carotenoids from Florida Valencia orange juice powders. Freshly prepared ————————; conditioned — — — — —; aged – – – – –. System—Petroleum ether-benzene –87% methanol.

The solution from every other tube was diluted with acetone to 30 ml. and the depth of color was then measured in an Evelyn photoelectric colorimeter using filter 440. Further dilutions if necessary were made with acetone. The values obtained were converted to total carotenoids by the use of a standard curve obtained with β-carotene. This method was used to obtain approximate values for the total carotenoid content of the various tubes, since the bulk of the carotenoids of oranges have spectral absorption maxima close to those of β-carotene. Some constituents, such as phytoene, phytofluene, ζ-carotene, auroxanthins, valenciaxanthin and sinensiaxanthin give very low or zero values by this method.

On the basis of color measurements (Figure 1) the contents of the tubes of the Craig apparatus were combined into 5 fractions. These were shown in earlier work *(1, 3)* to be: hydrocarbons + monols; diols; monoepoxide diols; diepoxide diols; and polyols. The 5 fractions, each with added absolute alcohol, were evaporated *in vacuo*. The residues were dissolved in benzene, except the hydrocarbon-monol fraction where petroleum ether was used.

Chromatography. The solutions, or aliquots of them, were then chromatographed on columns of magnesia (Westvaco No. 2642) plus filter aid (1:1 by volume or 1:2.3 by weight, respectively). The bands were eluted from the column by a graded series of eluents; the eluents were changed at intervals depending on rate of separation and elution of the various bands. For the diols, monoepoxide diols, and diepoxide diols, the eluents were 3.5%, 5%, 7%, 10%, 15%, and 25% ethanol in petroleum ether. The same series was used for the polyols starting with the 7% solution. In order to eluate the final fractions from the diepoxide diol and polyol fractions, 20% ethanol in benzene was used. For the hydrocarbon-monol fraction the eluents were successively 14%, 20%, 30%, 45%, and 70% benzene in petroleum ether and then benzene (these elute the hydrocarbons), followed by 0.5%, 0.7%, 1%, 1.4%, and 2% ethanol in petroleum ether (the latter elute the monols).

Each band eluted was collected separately, the solution was evaporated *in vacuo*, the residue dissolved in a measured volume of petroleum ether (hydrocarbons and monols) or benzene, and curves were run in a Cary recording spectrophotometer. The total absorbance of each constituent at its principal maximum was then calculated (the values for trans- and cis-isomers of a given carotenoid were combined, also other stereoisomers such as mutatoxanthins *a* and *b*, and luteoxanthins *a* and *b*) and from these values the total absorbance of all constituents and the percentage of each constituent. No correction was made for the different values of the specific absorbances of the various constituents because most of these values are unknown. The known values are of similar magnitude.

In addition to the sources of error or loss given above, it is probable that small losses occurred due to atmospheric oxidation during the various procedures. All operations were carried out in dim light and all solutions kept at 45° F. or lower whenever possible. The results obtained by the above procedures are obviously not strictly quantitative; hence small or moderate variations are of little significance. The results do show the relative abundance of the various constituents, and the major changes that occurred during the conditioning and aging processes.

RESULTS AND DISCUSSION

Single-strength juice. The content of total carotenoids (measured colorimetrically and calculated as β-carotene) after saponification in this very pulpy juice was about 16 mg. per liter. The distribution of the saponified carotenoids from the single-strength juice (Table 1) was similar to that of a similar preparation from California Valencia orange juice *(1)*. The components obtained on chromatography (Table 2) were also quite similar *(1, 2, 3)*, with two minor additions. The first, minor band eluted from the monoepoxide diol fraction on chromatography had absorption maxima in benzene of 479, 448 and 422 mμ, which are almost the same values as a mono-cis violaxanthin *(3)*. It was tentatively identified as a cis-lutein-5,6-epoxide. Another component from the monoepoxide diol fraction had spectral absorption maxima in benzene of 461, 433 and 409 mμ. After treatment with hydrochloric acid in methanol *(3)*, the maxima were at 459, 433 and 409 mμ. This was tentatively identified as flavoxanthin (lutein 5,8-epoxide), not previously found in orange juice carotenoids. If this band had been a luteoxanthin [diepoxide diols *(3)* which have similar absorption maxima], these maxima would have been shifted to those of auroxanthin (436, 411, 389 mμ).

Concentrated juice. The total carotenoid content, measured colorimetrically after saponification, of the 6-fold concentrated juice was 39 mg. per kg., equivalent to about 8 mg. per liter of single-strength juice. The distribution of the carotenoids (Table 1) was similar to that of the single-strength juice. The composition of the carotenoid mixture (Table 2) was similar to that from the single-strength juice; there was a somewhat higher ratio of 5,6-epoxide to 5,8-epoxide in the concentrate carotenoids than in those from the single-strength juice, indicating that no significant change in the carotenoid fraction had taken place during the concentration process, which is generally carried out at a low temperature, sometimes even below room temperature. Violaxanthin (zeaxanthin 5,6,5′,6′-diepoxide) is one of the most labile of the carotenoids and is isomerized in the presence of acid to luteoxanthins (zeaxanthin 5,6,5′,8′-diepoxides) and then to auroxanthins (zeaxanthin 5,8,5′,8′-epoxides). The relative amounts of these substances is an index to the extent of contact of the carotenoids with acid.

TABLE 1

Countercurrent distribution of carotenoids from Florida Valencia orange juice in benzene-petroleum ether—87% methanol

Fraction	Percentage of total carotenoids [1]					Ratio to amount found in freshly prepared powder	
	Single strength	Concentrate	Fresh powder	Conditioned powder	Aged powder	Conditioned	Aged
Hydrocarbon-Monol	14	13	14	18	23	1.03	0.97
Diol	21	19	21	27	29	1.01	0.82
Monoepoxide Diol	24	27	26	30	30	0.89	0.68
Diepoxide Diol	26	29	27	11	6	0.32	0.12
Polyol	14	12	12	14	13	0.85	0.61
					Entire sample	0.78	0.59

[1] Measured colorimetrically with 440 mμ filter.

TABLE 2

Carotenoid composition of Florida Valencia orange juices

Constituent	Approximate per cent of carotenoid mixture [1]					Ratio to amount found in freshly prepared powder	
	Single strength	Concentrate	Fresh powder	Conditioned powder	Aged powder	Conditioned	Aged
Phytoene	1.4	....[2]	2.1	2.5	3.2	1.02	1.00
Phytofluene	0.6	0.6	0.6	0.8	0.9	1.08	0.98
Alpha-carotene	1.0	1.4	1.1	1.5	1.7	1.09	1.01
Beta-carotene	1.2	1.8	1.6	2.1	2.4	1.11	0.98
Zeta-carotene	1.1	1.5	1.7	2.2	2.4	1.07	0.91
Gamma-carotene-like	0	0.2	0.2	0.2	0.5[3]	0.88	....
Hydroxy-alpha-carotene	2.9	2.8	2.5	2.5	2.8	0.85	0.73
Cryptoxanthin	5.4	5.6	4.4	4.9	6.6	0.93	0.98
Lutein	7.1	7.1	7.2	8.7	9.2	1.03	0.85
Zeaxanthin	9.2	9.0	8.3	9.9	11.3	1.01	0.90
Lutein 5,6-epoxide	0.3	0.3	0.3	0	0.04	0	0.10
Antheraxanthin	11.6	13.4	12.2	5.4	0.7	0.38	0.007
Flavoxanthin	0.14	0.3	0	0.3	0.4	....	....
Mutatoxanthins	7.3	8.0	9.4	16.7	22.9	1.50	1.63
Violaxanthin	14.7	18.8	13.6	1.5	0	0.09	0
Luteoxanthins	12.7	11.5	15.0	10.6	0.7	0.60	0.03
Auroxanthins	3.9	2.8	5.0	14.8	19.7	2.50	2.63
Valenciaxanthin	1.8	1.5	1.5	1.3	0.14	0.73	0.06
Sinensiaxanthin	1.6	1.6	1.5	0.4	0	0.24	0
Trollixanthin-like	10.4	7.6	7.1	6.1	0	0.73	0
Trollein	....[4]	....[4]	....[4]	....[4]	4.4	....	....
Valenciachromes	0.5	0.2	0.9	1.6	3.1	1.46	2.20
Trollichrome-like	3.4	1.7	2.1	4.5	6.4	1.87	1.99
Sinensiachrome	0.8	0.5	0.4	0.8	0	1.72	0
					Entire sample	0.91	0.72

[1] Calculated from sum of adsorbances at principal maximum on Cary spectrophotometer curve for each constituent.
[2] Probably present but obscured by impurity.
[3] Spectrophotometer curve indicated band was quite impure.
[4] Probably present but obscured by trollixanthin-like pigment.

One very minor component was found which had not been found previously in orange juice carotenoids. It occurred in the column between ζ-carotene and hydroxy-α-carotene and had spectral absorption maxima in petroleum ether of 459 and 436, with a pronounced shoulder at 489 mμ. These values are considerably higher than those of β-carotene and are a few mμ below those of γ-carotene. This fraction may be impure γ-carotene, or a cis-isomer or mixture of isomers thereof. Due to the small amount of carotenoid present it was not further investigated.

Powdered juice. As stated above, the powdered juice was prepared from the concentrate, with some added pulp from the single-strength juice. A small amount of carotenoids was also present in the cold-pressed orange oil added to the finished powder. The total carotenoid content, after saponification, was about 61 mg. per kg. (measured colorimetrically). The distribution of the carotenoids (Figure 1, Table 1) was similar to that of the concentrate carotenoids. The percentages of the individual carotenoids (Table 2) were in most cases close to those of the carotenoids from the concentrate. The acid-catalyzed 5,6-epoxide-5,8-epoxide change had occurred to a minor degree, the percentages of antheraxanthin and violaxanthin decreasing and those of the mutatoxanthins, luteoxanthins, and auroxanthins increasing. On the whole the changes occurring in the powder preparation were minor.

Conditioned powder. The total carotenoids, measured colorimetrically after saponification, amounted to about 47 mg. per kg., a decrease of 22% during the conditioning period. The distribution (Figure 1, Table 1) showed little change in the hydrocarbon-monol and diol fractions, moderate decreases in the monoepoxide diol and polyol fractions, and a large decrease in the diepoxide diol fraction. The increases (Table 1) in the percentages (based on total carotenoids) of all of the other fractions was due to this large decrease. Table 2 shows that the 5,6-epoxide-5,8-

epoxide isomerization had proceeded to a considerable extent, well over half in the case of antheraxanthin and 90% for violaxanthin. No significant changes had occurred in the non-epoxide carotenoids.

Aged powder. The total carotenoids when measured colorimetrically after saponification had declined to 36 mg. per kg., a decrease of 41% based on the fresh powder. The distribution (Figure 1, Table 1) now showed little change in the hydrocarbon-monol fraction, and a decrease of around 20% for the remainder, when compared with the conditioned powder carotenoids. The shift in the positions of the maxima in Figure 1 was due to the use of an automatic filling device which delivered about 11 ml., instead of the usual 10 ml., so that the upper layer in each tube was larger, and the fractions were moved along a few tubes further in the Craig apparatus. Figure 1 for the aged powder is similar to the curve obtained for the carotenoids of aged canned Valencia orange juice (single-strength, stored at room temperature for 3 years) *(4)*.

Table 2 shows that the 5,6-epoxides (antheraxanthin, violaxanthin, luteoxanthins, lutein 5,6-epoxide, valenciaxanthin, sinensiaxanthin, trollixanthin-like) had practically all disappeared. Increases were found in all of the corresponding 5,8-epoxides except sinensiachrome, none of which was found. The increases in 5,8-epoxides, in particular in the mutatoxanthins and auroxanthins, were considerably less than the decreases in the corresponding 5,6-epoxides. This might mean that a part of the 5,8-epoxides disappeared during the aging process, or else the residual 5,6-epoxides had decomposed to form substances, perhaps colorless, other than the 5,8-epoxides. Trollein *(3)* appeared for the first time in the aged juice when the trollixanthin-like substance had practically all disappeared. These two substances are separated poorly under the conditions used for chromatography.

It is noteworthy that the three provitamins A, α- and β-carotenes and cryptoxanthin, were found in practically unchanged amounts at the end of the aging period.

Table 1 shows that in the conditioning and aging processes there were decreases in the total carotenoids (when measured colorimetrically with a 440 mμ filter) of 22 and 19%, respectively, whereas in Table 2 where the contents of the individual carotenoids were measured spectrophotometrically, the decreases in total carotenoids were 9 and 19%, respectively, for the 2 periods. The difference between the values of 22 and 9% for the 2 methods during the conditioning period is probably due largely to the isomerization of violaxanthin and the luteoxanthins to auroxanthins. The absorption maxima of the latter (427, 402 and 381 mμ in ethanol) are at wave lengths below that of the 440 mμ filter used in the Evelyn colorimeter, with the result that solutions of auroxanthins give much lower values when measured colorimetrically. The changes with violaxanthin, a 5,6,5′,6′-diepoxide, are much more pronounced than for the other 5,6-epoxides which are all monoepoxides.

The specific absorbances of carotenoids generally decrease with decrease in wave length of the spectral absorption maxima. 5,6-expoxides generally have higher specific (or molar) absorbances than the corresponding 5,8-epoxides, hence this isomerization results in an apparent loss of total carotenoids when measured spectrophotometrically.

In many cases, in the carotenoids from the aged juice, the spectral absorption maxima occurred at 1 or 2 mμ shorter wave length than in the corresponding fractions from fresh powder. This indicates that considerable trans-cis isomerization occurred during the conditioning and aging processes. Cis-isomers generally have lower specific absorbances than the corresponding all-trans isomers. This trans-cis isomerization may have been responsible, at least in part, for the moderate decreases observed for a number of carotenoids, such as ζ-carotene, hydroxy-α-carotene, lutein and zeaxanthin.

Thus, the 5,6-epoxide-5,8-epoxide and trans-cis isomerizations are probably responsible for a considerable part of the apparent losses of either total carotenoids measured colorimetrically or of individual carotenoids measured spectrophotometrically, which occur on conditioning and aging of orange juice powder. Other reactions resulting in the formation of colorless products may be responsible for a part of the apparent losses.

SUMMARY

A study was made of the changes in the carotenoid fraction during the preparation and storage of powdered Florida Valencia orange juice. The composition of the carotenoid mixtures from frozen single-strength and concentrated juices showed no significant differences indicating that the concentration process had little or no effect. In the preparation of powdered orange juice a minor part of the carotenoid 5,6-epoxides, which compose about ⅔ of the pigments, was isomerized to 5,8-epoxides. This change occurred to a much greater extent on conditioning the powder at 77° F. for 78 days. On subsequent storage at 100° F. for 6 months, the 5,6-epoxides practically all disappeared and appreciable decreases were found in some of the non-epoxide carotenoids, which may be, at least in part, accounted for by trans-cis isomerization. Most of the apparent decrease in total carotenoid content, when measured colorimetrically at 440 mμ or spectrophotometrically for individual constituents can be accounted for by these isomerizations. No losses were observed in the provitamins A (cryptoxanthin and β- and α-carotenes) during the conditioning and aging periods.

The composition of the carotenoid mixture from Florida and California Valencia orange juices was similar, though the total content was lower in the Florida juice. Two very minor components obtained from Florida juice which had not previously been found in California juice, were apparently lutein 5,6-epoxide and the corresponding 5,8-epoxide flavoxanthin.

Acknowledgment

The orange juice powders used in this study were prepared by George K. Notter and Doris H. Taylor of this Laboratory.

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Storage Characteristics of Some Irradiated Foods Held at Various Temperatures Above Freezing[a,b,c]

I. Studies with Chicken Meat and Sweet Potatoes

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Although many investigators have reported the immediate effects induced in various foods treated with ionizing radiations, little information is available concerning the behavior of irradiated foods during storage at different temperatures. The objective of this investigation was, therefore, to observe and study the storage characteristics of a variety of irradiation-sterilized meats, fish and vegetables held at temperatures ranging from 50° F. to 125° F. These food items included beefsteak, chicken, hamburg, fresh pork sausage, halibut steaks, scallops, green beans, spinach, and sweet potatoes. The selection of these particular products was based, first of all, on their ability to withstand sterilizing doses of ionizing radiations without extreme adverse effects, as shown by earlier experiments, and second, on their popularity as foods.

In this paper, only the results obtained with chicken and sweet potatoes will be discussed.

EXPERIMENTAL METHODS

I. Preparation of Food Products for Storage Studies

The container used for food samples that were irradiated or otherwise treated for storage studies was a quarter-pound sardine can, lined with A-enamel in the case of chicken, and unlined in the case of sweet potatoes. To minimize oxidative changes during storage, the cans were sealed under a vacuum of about 25 inches of mercury by means of a semi-automatic American Can Company can-sealer.

Chicken. Birds that had been caponized by hormone treatment were slaughtered, plucked by the semi-scald method, and eviscerated. The birds were held at a temperature of 36–40° F. for 18–24 hours before processing so that they might be dissected into such sections as legs, breasts, etc., after the passage of rigor mortis; these sections were blanched in live steam (212° F.) for 6 minutes and cooled under running tap water. The formation of a pink color after irradiation had been noted in preliminary experiments with chicken meat. Blanching before irradiation prevented this to some extent and also facilitated the separation of the meat from the bone during preparation for the irradiation treatment. It was also considered that blanching might be desirable as a means of inactivating proteolytic enzymes. The blanched meat was removed from the bone, white and dark meats kept separate, and packed under vacuum in sardine cans. Some samples were frozen at 0° F. to serve as frozen controls, some were heat-processed, and some were irradiated in the frozen state with a dose of 3×10^6 rep.

Since the product had to be irradiated from both sides of

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[a] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May 28, 1958.

[b] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 922 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

[c] Contribution No. 355 from the Department of Food Technology.

the can in order to achieve sufficient penetration, the sample was irradiated in the frozen state so that the mass of meat would remain intact when the can was turned over.

Heat-processed samples were prepared to serve as reference controls in the taste tests. Heat penetration data were obtained by thermocouple measurement; the processing time with respect to an F_0 value of 5.0 was determined by the graphical method.

Some of the irradiated samples were held at each of the following storage temperatures: $50 \pm 2°$ F., $68 \pm 2°$ F., $85 \pm 2°$ F., $99 \pm 2°$ F., and $125 \pm 2°$ F. Heat-processed samples were stored at $68 \pm 2°$ F. These conditions of storage were also used for other irradiated or heat-processed food in this investigation.

Sweet potatoes. The tubers were cooked in live steam for about 25 minutes and then cooled under water, peeled, and puréed. The puréed product was vacuum packed in plain-tin sardine cans. Some samples were heat-processed, some were frozen at 0° F., and others were irradiated with a dose of 2.5×10^6 rep.

Storage conditions for the different samples were the same as those indicated for chicken.

In preliminary experiments with sweet potatoes, a darkening of the product occurred when an enameled container was used. However, this condition was obviated through the use of plain-tin cans. It is presumed that sufficient tin dissolved to maintain reducing conditions in the product.

II. Bacteriological Methods

Standard plate counts for viable aerobic microorganisms were made according to standard methods of analysis *(4)*. The initial dilution was prepared by disintegrating 22 g. of the product with 198 ml. of chilled, sterile, distilled water in a sterile Osterizer for 3 to 5 minutes, depending on the nature of the product. Appropriate decimal dilutions were made and plated in duplicate on tryptone-glucose extract agar. Plate cultures were incubated at 30° C. for 48 to 72 hours before colonies were counted.

The Most Probable Numbers (MPN) of anaerobic organisms were determined by inoculating a series of 5 culture tubes containing sterile thioglycollate broth medium with 1.0 ml. portions of an appropriate dilution of product and stratifying these cultures with sterile, melted vaseline. This procedure was then repeated for two successively greater dilutions. A two week incubation period at 37° C. was allowed after which tubes showing gas production and growth, or growth alone, were noted and counts of putrefactive and total anaerobes were calculated from Most Probable Numbers tables *(5)*.

III. Chemical and Physical Tests

A. Amino nitrogen content. The free amino nitrogen content of irradiated and non-irradiated foods was determined by the method of Pope and Stevens *(8)*. This test was conducted in order to detect proteolytic enzyme activity as indicated by the extent of amino acid formation during storage at the various temperatures.

B. Oxidation of fats. The course of fat oxidation of dark meat of chicken during storage at the different temperatures was followed by measuring the peroxide number *(14)* and thiobarbituric acid value (TBA) *(11)*.

C. Vitamin content. *Carotene.* Determinations for β-carotene were made according to a modified method currently in use in the Department of Food Technology, M.I.T. *(1)*. Essentially, the procedure involves digestion with alcoholic potassium hydroxide solution, extraction of the vitamin with petroleum ether, adsorption on a Celite-MgO column, elution with 5% acetone in petroleum ether, and measurement of the carotene content by means of an Evelyn Photoelectric Colorimeter with reference to a standard curve prepared from pure β-carotene.

Pyridoxine. Pyridoxine was assayed by a microbiological method using the pyridoxineless mutant Number 299 of *Neurospora sitophila*. The weight of mycelium produced when the organism was grown in a basal medium containing a certain amount of test sample was compared with the weight of mycelium produced when the organism was grown in basal media containing variable amounts of pyridoxine *(2)*.

Riboflavin. Riboflavin determinations were carried out by a modified method that is used in the Department of Food Technology *(3)*. The procedure consists essentially of the extraction of the vitamin with 0.1 *N* sulfuric acid, followed by treatment with takadiastase and adsorption on a Florisil column followed by elution with 20% pyridine-acetic acid solution, and the reading of the fluorescence in a photofluorometer. An internal standard was used.

Thiamin. Thiamin was measured according to the method of the Association of Vitamin Chemists *(10)*. Basically, the procedure consists of the extraction of the vitamin with 0.1 *N* sulfuric acid, conversion of the vitamin to thiochrome and measurement of the fluorescence in a photofluorometer.

D. Color measurement. Color changes induced in the foods as a result of irradiation or heat-processing and also the color changes occurring during storage at various temperatures, were measured with an American Optical Rapid Scanning Spectrophotometer, equipped with a reflectance attachment. The instrument was standardized with a didymium filter and then adjusted to 0 and 100% reflectance. The spectral reflectance curve of the sample, which appeared on the face of the oscilloscope tube, was photographed with a Fairchild Polaroid camera.

IV. Organoleptic Evaluation and Statistical Analysis

Two small panels consisting of six judges were employed for the flavor evaluation of the various irradiated and stored products. Each judge was given a score sheet which had a range of numerical values from one to ten with descriptive terms also included. These were as follows:

10	Excellent; appetizing; tempting
9 to 8	Good; pleasing; palatable
7 to 6	Fair; indifferent; flat; slightly off-flavor
5 to 4	Poor; unappetizing
3 to 2	Very poor; objectionable
1	Bad; very unpleasant

The design of the organoleptic test was based on a randomized incomplete block *(6)*. The plant included 6 treatments, 5 replications, 10 blocks or tests and 3 variations per test. With increasing storage time, however, the number of treatments was reduced due to the elimination from the tests of the irradiated samples held at the high storage temperatures.

Statistical significance among treatments was determined by analysis of variance *(9)* and Duncan's Multiple Range Test *(7)* was employed to locate differences within groups.

Chicken and sweet potatoes were prepared for tasting by heating the unopened cans in boiling water for 15 minutes. These products were served without any seasoning.

V. Irradiation Techniques

A Van de Graaff accelerator *(12)* rated at 3 M.e.v. was the source of high-voltage cathode rays for the irradiation of the sweet potatoes and a unit with a 2 M.e.v. potential (High Voltage Engineering Corporation, Burlington, Mass.) was used for the chicken irradiation.

Samples to be irradiated were placed on a motor driven belt, the path of which traversed the electron beam. For foods packed in sardine cans, irradiation from both sides through the shortest dimension of the can was required. Under these conditions the proper thickness (unit density material) was calculated to be about 17 mm. when a 3 M.e.v. beam was applied, and 11 mm. when a two M.e.v. beam was used.

The requested dose was checked by ferrous-ferric dosimetry *(13)*. A run was considered satisfactory if the requested dose and calculated dose did not differ by more than 10%.

RESULTS AND DISCUSSION

Throughout storage at any of the temperatures for either irradiated chicken meat or sweet potatoes, the standard plate count and most probable number of anaerobes remained at an insignificant level, and therefore, commercial sterility was attained in each product with the particular irradiation dose applied.

Dark meat. Figure 1 shows the graphs which resulted when mean flavor scores of the frozen control and irradiated samples of dark meat of chicken stored at various temperatures were plotted against storage time. In some instances the mean flavor scores fluctuated from one month to another for no explicable reason other than sampling variation or variability due to panel participants. Therefore, the curves as drawn represent the trend that the mean flavor scores appeared to follow with storage time at a given temperature.

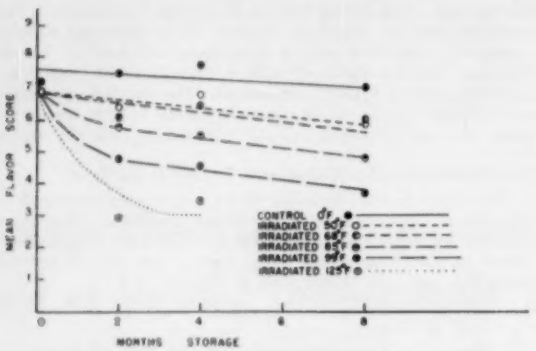

Figure 1. Mean flavor score of irradiated (3 x 10⁶ rep) and non-irradiated dark meat of chicken held at various temperatures.

According to the design of the flavor testing, a score of 5.5 was regarded as the dividing line between acceptability and unacceptability. From Figure 1 it can be seen that irradiated dark meat samples held at either 50° F. or 68° F. were still acceptable after eight months of storage. Samples stored at 85° F. remained acceptable for 4 months, whereas samples kept at 99° F. or 125° F. became unacceptable after one month or less.

There was no significant difference (5% level) between the flavor of irradiated and non-irradiated dark meat samples immediately following the irradiation treatment, but throughout an 8-month storage period the frozen controls were found to be significantly different in flavor compared with irradiated samples held at any of the given temperatures. There was no significant flavor difference between irradiated dark meat samples held at 50° F. and those held at 68° F., but when irradiated samples held at either of these temperatures were compared with irradiated samples held at the higher temperatures (85° F., 99° F., or 125° F.), significant differences were observed. After 4 months of storage, irradiated dark meat samples held at 85° F. or higher had undergone proteolysis to some extent and as a result had acquired a soft texture. A bitter flavor was also apparent. This same bitter flavor could be detected to a slight degree among irradiated samples held at 68° F. after 8 months. The free amino nitrogen content as a function of storage time of irradiated and non-irradiated dark meat chicken held at the various temperatures is shown in Figure 2. The free amino nitrogen content

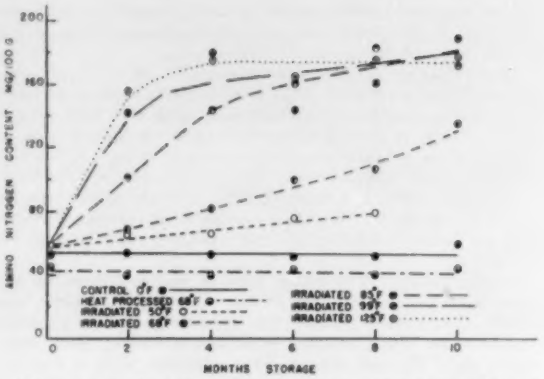

Figure 2. Amino nitrogen content of irradiated (3 x 10⁶ rep) and non-irradiated dark meat of chicken held at various temperatures.

of the frozen controls or heat-processed samples remained relatively constant over a 10-month period. The irradiated samples showed an increase in free amino nitrogen and the rate of amino nitrogen formation was generally more rapid during the early part of storage and also increased with increasing storage temperatures.

Peroxide values (Table 1) of irradiated or unirradiated samples of dark meat generally remained at zero or thereabouts during storage. This would seem to indicate that fat oxidation was not occurring. However, after 6 to 8 months' storage the taste panel could detect a slightly rancid flavor in the frozen controls and in the irradiated samples held at 50° F. or 68° F. The thiobarbituric acid values did not appear to follow any definite trend, but seemed to indicate that there was some variability in the fat of the birds used in this study. Of the irradiated samples, those held at the lower temperatures seemed to have the highest TBA value.

At the beginning of storage the controls and irradiated samples were practically indistinguishable in color, both being brownish-pink. After 8 months of storage the color of the frozen control was still about the same, whereas the irradiated samples had taken on more of a pinkish hue which was less intense in the samples stored at the higher temperatures. The reflectance curves are presented in Figure 3.

White meat. The mean flavor scores, as a function of storage time, of irradiated white meat of chicken held at different temperatures are shown graphically in Figure 4.

Irradiated samples held at 50° F. or 68° F. were still regarded as acceptable following 10 months of storage. Irradiated samples stored at 85° F. remained acceptable for 4 months, whereas when the storage temperature was 99° F. or 125° F. they became unacceptable in less than 2 months.

Immediately after irradiation there was no significant flavor difference (5% level) between irradiated and unirradiated samples. Over a 10-month storage period the frozen controls were not significantly different flavorwise from irradiated samples held at

TABLE 1

Peroxide number and thiobarbituric acid value (TBA) of irradiated (3 x 10^6 rep), non-irradiated and heat processed chicken meat held at different temperatures

Product	Storage time (months)	Chemical test	Control 0° F.	68° F. heat process	50° F. Irrad.	68° F. Irrad.	85° F. Irrad.	99° F. Irrad.	125° F. Irrad.
Chicken (dark meat)	0	Peroxide No.[1]	0	0	0	0	0	0	0
		TBA value[2]	2.14	2.04	2.34	2.34	2.34	2.34	2.34
	2	Peroxide No.	0	0	0	0	0	0	0
		TBA value	0.855	0.901	2.27	0.731	0.452	0.453	0.441
	4	Peroxide No.	8	0	0	0	0	0	0
		TBA value	2.226	1.589	1.032	0.712	0.467	0.578	0.381
	6	Peroxide No.	2	0	1.1	0	0	0	0
		TBA value	1.33	0.69	1.44	0.67	1.02	0.68	0.44
	8	Peroxide No.	0	0	0	0	0	0	0
		TBA value	1.07	0.97	1.16	0.56	0.52	0.26	
	10	Peroxide No.	0	0	0	0	0	0	
		TBA value	0.65	0.67	0.71	0.51	0.49	0.76	

[1] = millimoles/kg. fat.
[2] = optical density.

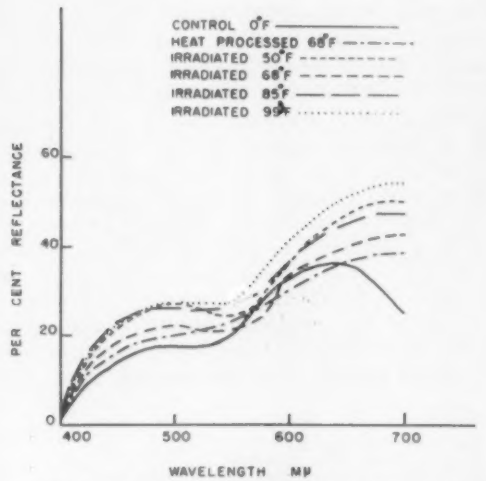

Figure 3. Reflectance curves for irradiated (3 x 10^6 rep) and non-irradiated dark meat of chicken held for eight months at various temperatures.

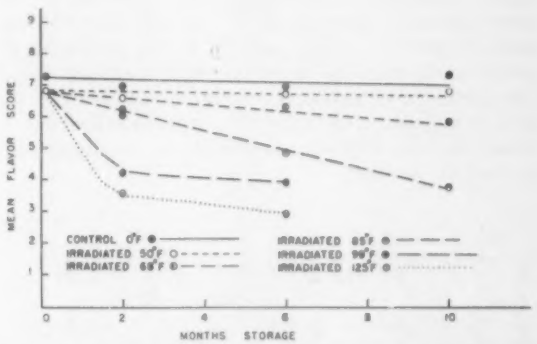

Figure 4. Mean flavor score of irradiated (3 x 10^6 rep) and non-irradiated white meat of chicken held at various temperatures.

50° F., but when compared with irradiated samples held at 68° F. to 125° F., there was a significant difference. After 2 months a bitter and acidic taste was detected in irradiated samples held at 99° F. or 125° F., and texture changes were also evident. This was probably due to the action of proteolytic enzymes. This same bitter off-flavor became apparent in irradiated samples held at 85° F. after 6 months' storage and in irradiated samples held at 68° F. after 10 months, but was never detected in irradiated samples held at 50° F. This bitter flavor could be attributed to the breakdown products of proteolysis or to reaction products resulting from non-enzymatic browning since irradiated samples held at the higher temperatures were observed to have undergone browning to a certain extent.

At the start of storage there was scarcely any color difference between the controls and the irradiated samples, both being a pale shade of cream. The re-

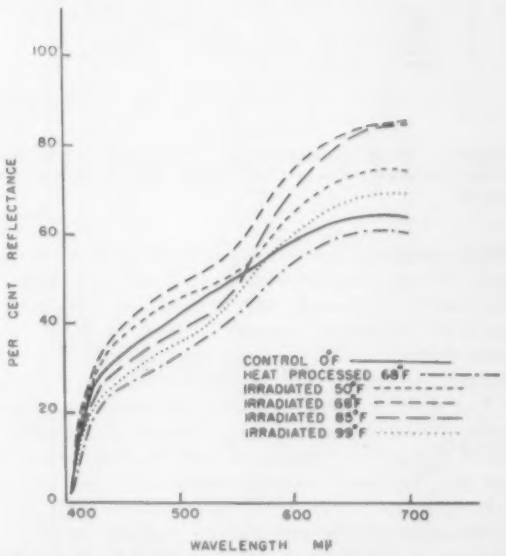

Figure 5. Reflectance curves for irradiated (3 x 10^6 rep) and non-irradiated white meat of chicken held for ten months at various temperatures.

flectance curves of the 2 different samples were almost identical. After storage for 10 months the frozen controls had darkened slightly and the irradiated samples varied in color from a light, pinkish-tan at 50° F. storage to an orange-tan at 99° F. storage. Heat-processed samples retained about the same tan color throughout storage. Reflectance curves for 10-month samples can be seen in Figure 5.

The free amino nitrogen contents of the variously stored irradiated samples are shown in Figure 6. As occurred with irradiated dark meat, the irradiated white meat samples showed an increase in free amino nitrogen and the rate of amino nitrogen formation was generally more rapid during the early part of storage and increased as the storage temperature increased. At the higher storage temperatures (85° F. to 125° F.), amino nitrogen in irradiated samples remained constant or even declined after approximately 4 months. Since the samples held at these higher temperatures were observed to have undergone non-enzymatic browning, it is probable that the free amino acids were being used up by the browning reaction at an equal or faster rate than they were being liberated. Nevertheless, these graphs indicate that the heat treatment given to the chicken meat before irradiation was not sufficient to completely inactivate the proteolytic enzyme.

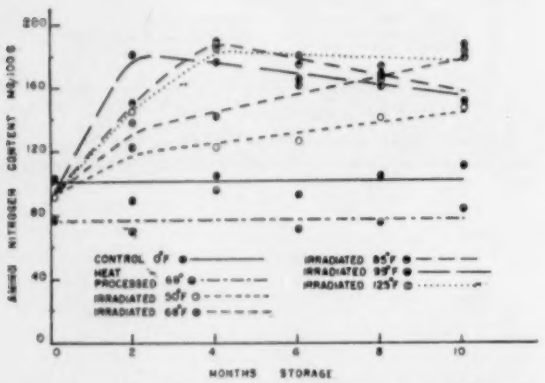

Figure 6. Amino nitrogen content of irradiated (3×10^6 rep) and non-irradiated white meat of chicken held at various temperatures.

Sweet potatoes. No significant flavor difference was found between irradiated and non-irradiated samples immediately following the irradiation treatment. Even after 10 months' storage at 50° F. or 68° F., the frozen controls and irradiated samples were not significantly different. The irradiated samples held at 50° F. and 68° F. were consistently considered to have a better flavor than the frozen controls throughout 8 months of storage. During storage at other temperatures the irradiated samples remained "not significantly different" from the frozen controls until after the following storage periods had elapsed: at 85° F., 8 months; at 99° F., 4 months; at 125° F., 2 months.

The approximate storage lives of irradiated sweet potatoes at various holding temperatures may be estimated from Figure 7. Thus, at 50° F. or 68° F. the irradiated samples remained acceptable even after being stored for 10 months. At 85° F., the irradiated product became unacceptable after 7 months. Irradiated samples withstood a 99° F. storage temperature for about 5 months before becoming unacceptable and at 125° F. storage life was approximately one month.

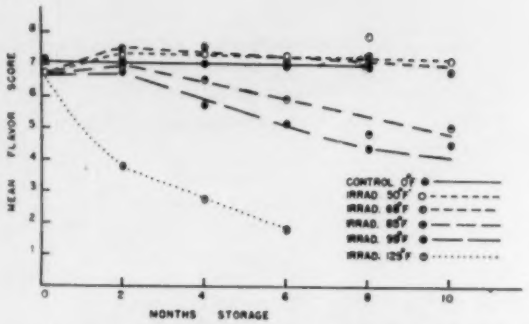

Figure 7. Mean flavor score of irradiated (2.5×10^6 rep) and non-irradiated sweet potatoes held at various temperatures.

The factor mostly responsible for the loss of acceptability of irradiated samples during storage was internal corrosion of the cans, which imparted a metallic flavor to the product. However, samples held at 125° F. darkened considerably and this probably affected the acceptability to some extent. Sweet potatoes were canned in unlacquered sardine cans which had been tinned by the electrolytic process. Corrosion of the container by irradiated sweet potatoes could probably be delayed by using a can from tin plate with a higher amount of tin per base box.

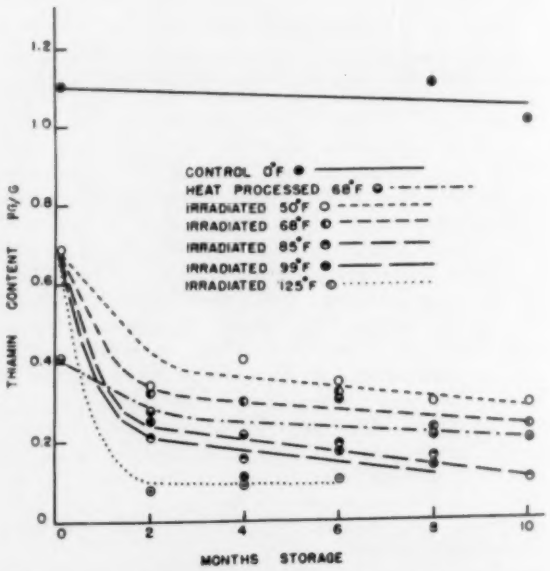

Figure 8. Thiamin content of irradiated (3×10^6 rep) and non-irradiated white meat of chicken held at various temperatures.

During storage at the various temperatures, the free amino nitrogen content of irradiated samples remained relatively constant (data not shown) and this indicated that no proteolytic degradation was occurring and that darkening of samples held at 125° F. was probably not due to amino acid-carbonyl condensations.

At the beginning of storage the irradiated and unirradiated samples were quite similar in color. After 10 months of storage at various temperatures, there was scarcely any difference in the color of irradiated samples held from 50° F. to 99° F. These samples all possessed a bright orange color which did not appear to have changed significantly since the start of storage. The frozen controls, however, had darkened somewhat during storage and generally showed less reflectance in the orange region of the visible spectrum than did the irradiated samples.

Stability of certain vitamins in irradiated products held at various storage temperatures. The thiamin, riboflavin, and pyridoxine contents of irradiated and non-irradiated white meat of chicken, and the carotene content of irradiated and non-irradiated sweet potatoes are presented graphically in Figures 8 through 11 as a function of storage time at various temperatures. Of the vitamins considered in this study, pyridoxine was the most resistant to inactivation by cathode rays, the resulting loss being only about 5–10% of the initial concentration. Also, in general, the heat-process treatment caused about the same destruction of these vitamins as did the irradiation treatment. Heat-processed sweet potatoes retained about 83% of the original carotene content whereas with irradiated samples there was only a 45% retention.

During storage the rate of vitamin destruction among irradiated samples was a function of temperature. Pyridoxine displayed the least stability during storage.

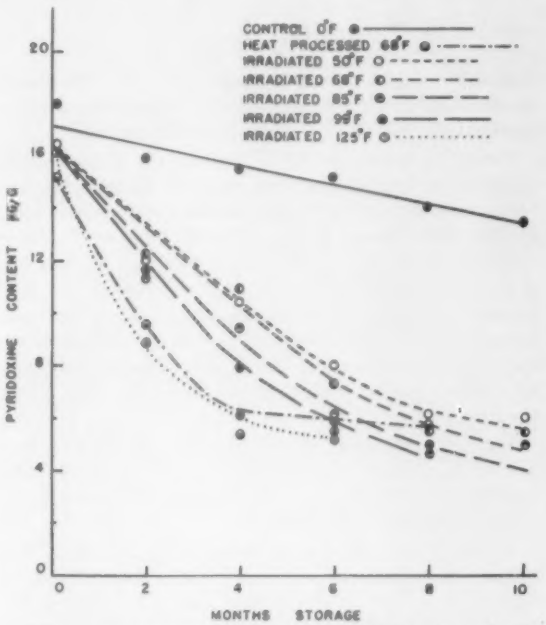

Figure 10. Pyridoxine content of irradiated (3 x 10^6 rep) and non-irradiated white meat of chicken held at various temperatures.

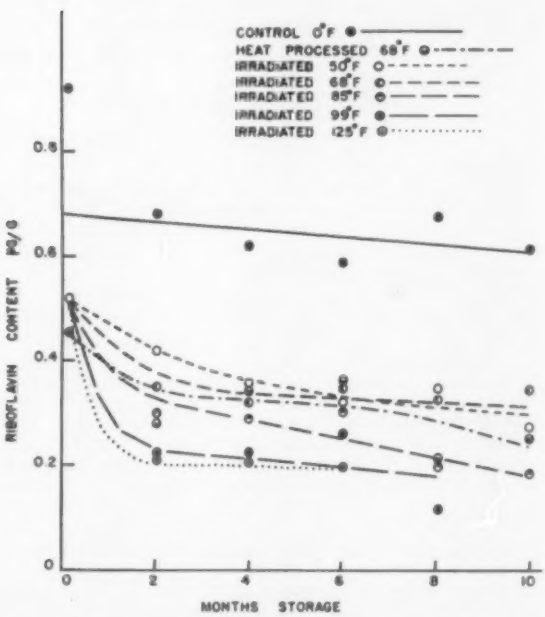

Figure 9. Riboflavin content of irradiated (3 x 10^6 rep) and non-irradiated white meat of chicken held at various temperatures.

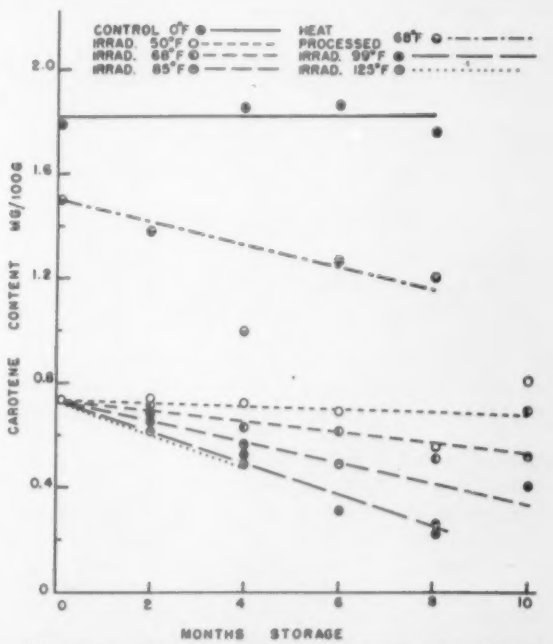

Figure 11. Carotene content of irradiated (2.5 x 10^6 rep) and non-irradiated sweet potatoes held at various temperatures.

There did not appear to be much difference in the rates of destruction of the various vitamins in heat-processed samples stored at 68° F. compared with irradiated samples stored at the same temperature. This can be interpreted as indicating that irradiated foods are not more susceptible to vitamin loss during storage than are conventional heat-processed foods.

SUMMARY

Storage studies were carried out with irradiated chicken and sweet potatoes held at temperatures ranging from 50° F. to 125° F. for 8 to 10 months.

Products stored at 50° F. and at 68° F. were still regarded as acceptable after eight to 10 months. Deterioration in quality occurred within one month when samples were stored at 125° F.

With irradiated chicken, shelf life was limited by: proteolytic enzyme action which resulted in poor texture; non-enzymatic browning, which caused undesirable color changes and presumably imparted a bitter off-flavor; and by the development of oxidative rancidity, particularly at the lower storage temperatures.

Internal corrosion of the metal container occurred during the storage of irradiated sweet potatoes. This produced a metallic flavor in the product and was the chief cause of loss of quality.

Vitamin analysis for thiamin, riboflavin and pyridoxine indicated that the amount of these vitamins destroyed by a sterilizing dose of cathode rays was comparable to the amount destroyed by conventional methods of thermal processing. Loss of vitamins in irradiated samples or heat-processed samples during storage at similar temperatures proceeded at about the same rate during storage.

Acknowledgment

The authors gratefully acknowledge the cooperation of J. G. Trump and K. A. Wright of the Department of Electrical Engineering, M.I.T., in the use of the 3 M.e.v. Van de Graaff accelerator.

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Storage Characteristics of Some Irradiated Foods Held at Various Temperatures Above Freezing

II. Studies with Pork Sausage and Scallops[a,b,c]

(Manuscript received May 31, 1958)

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In a previous paper the storage characteristics of irradiated chicken meat and sweet potatoes held at temperatures ranging from 50° F. to 125° F. were described *(1)*. This paper reports the observations of similar studies made with two other products, notably pork sausage and sea scallops.

Pork sausage and sea scallops are two food items which do not lend themselves particularly well to sterilization by conventional heat-processing methods. During heat-processing scallops change in color to dark brown due to non-enzymatic browning. Heat-processing causes denaturation of the hemoglobin pigment of pork sausage with subsequent darkening, and in addition, some of the fat is rendered from the product.

In earlier studies it was demonstrated that pork sausage and scallops could be sterilized with high-voltage cathode rays and still maintain the appearance of the normal fresh product; however, the behavior of these irradiation-sterilized products in storage was not known. This investigation was undertaken to determine the storage characteristics of irradiation-sterilized scallops and pork sausage.

EXPERIMENTAL METHODS

I. Preparation of Food Products for Storage Studies

All samples were packaged in quarter-pound sardine cans lined with A-enamel and were sealed under a vacuum of about 25 inches of mercury by means of a semi-automatic American Can Company sealer.

Pork sausage. Pork butts were cut up into cubes approximately one inch on each side and the cubed meat was thoroughly mixed with the following seasoning agents per 100 pounds of meat: Salt, 28 ounces; black pepper, 6 ounces; sage, 2 ounces.

The spiced meat was passed twice through a meat grinder, first with a ⅜ in. plate and finally, with a ⅛ in. plate. The sausage was rolled out into sheets 1 cm. thick and patties were stamped out by means of a sardine can body with a crimped lip. These were vacuum packed in sardine cans. Some samples were frozen at −20° F., some were heat-processed and stored at 68° F., and others were irradiated with a dose of 3.5×10^6 rep and held at storage temperatures of 50 ± 2° F., 68 ±2° F., 85 ± 2° F., 99 ± 2° F., 125 ± 2° F.

Scallops. Fresh sea scallops were sliced across the grain of the muscle in such a manner that discs 1 cm. thick were obtained. The sliced scallops were blanched in live steam (212° F.) for 4 minutes to inactivate proteolytic enzymes, and cooled in running tap water for 10 minutes to leach out reducing sugars or other components which might participate in non-enzymatic browning reactions. The blanched scallops were vacuum packed in sardine cans with 1% brine and frozen at 0° F. Some samples were irradiated in the frozen state (to prevent the product from moving when the can was inverted for purposes of irradiation), with a dose of 3×10^6 rep. Other samples were not irradiated.

The unirradiated samples were stored at 0° F. and the irradiated samples were stored at temperatures ranging from 50° F. to 125° F.

No heat-processed scallop samples were prepared due to the poor texture and color which results upon heat-processing this product.

II. Analytical Methods

Bacterial counts; assay procedures for thiamin, riboflavin and pyridoxine; tests for free amino nitrogen content as a measure of proteolysis; tests for fat oxidation; color measurement; and organoleptic evaluation have been described in a previous paper *(1)*.

III. Preparation of Samples for Flavor Testing

Scallops were rolled in flour and pan fried in a hydrogenated fat. Pork sausage patties were broiled in an electric oven. These products were served without any seasoning.

IV. Irradiation Technique

A two M.e.v. Van de Graaff accelerator was the source of the ionizing radiations. The reader is referred to a previous paper *(1)* for an adequate description of the irradiation procedure.

RESULTS AND DISCUSSION

An irradiation dose of three million rep in the case of scallops, and three and one-half million rep in the case of pork sausage rendered these products commercially sterile, since throughout an 8- to 10-month storage period at temperatures ranging from 50° F. to 125° F. the standard plate counts and most probable number of anaerobes remained at an insignificant level.

Pork sausage. Immediately following irradiation treatment, a significant flavor difference was noted between irradiated and non-irradiated pork sausage samples. Nevertheless, the flavor of the irradiated product was not objectionable since every judge rated the irradiated samples as acceptable.

The frozen controls remained significantly different in flavor compared with the irradiated samples throughout an 8-month storage period at the different temperatures. Irradiated samples held at 50° F. were

[a] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned Number 919 in the series of papers approved for publication.

[b] Contribution No. 356 from the Department of Food Technology.

[c] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May 28, 1958.

considered to be acceptable by a large majority of the judges, whereas the acceptability of the irradiated samples held at 68° F. or at higher temperatures was generally low.

When the graphs of mean flavor score as a function of storage time at various temperatures (Figure 1) are considered, it may be seen that irradiated sausage meat held at 50° F. was still considered acceptable after 8 months. Irradiated samples at 68° F. storage became unacceptable after approximately 2 months and with storage at 85° F. or above; irradiated pork sausage was regarded as not acceptable at the end of about one month.

These conclusions were based upon a mean flavor score of 5.5 as representing the line of demarcation between acceptability and unacceptability. Some of the factors which were responsible for the unacceptability of this product included an acidic or bitter flavor, loss of texture due to proteolytic enzyme action, and a rancid flavor. The effects of proteolysis were evident in irradiated samples held at 99° F. or 125° F. after 2 months; and in samples held at 68° F. following 8 months of storage. The free amino nitrogen contents of the samples stored at different temperatures during an 8-month period are indicated in Figure 2. The

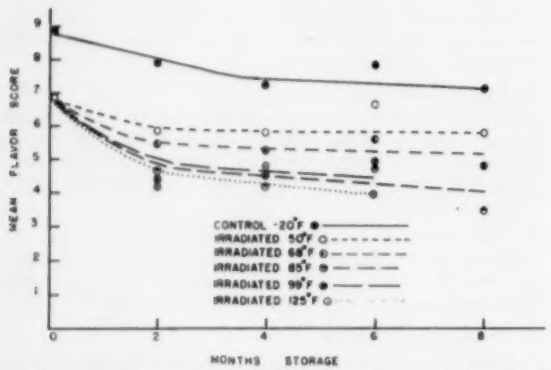

Figure 1. Mean flavor score of irradiated (3.5×10^6 rep) and non-irradiated pork sausage held at various temperatures.

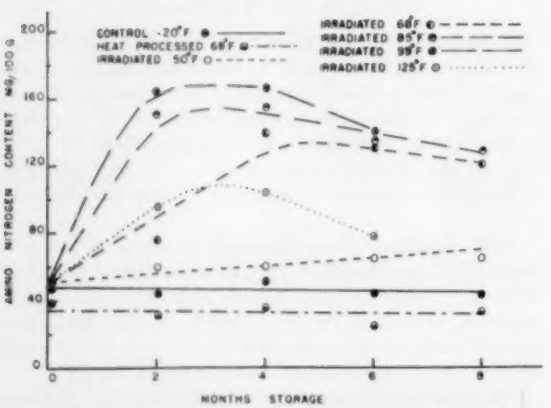

Figure 2. Amino nitrogen content of irradiated (3.5×10^6 rep) and non-irradiated pork sausage held at various temperatures.

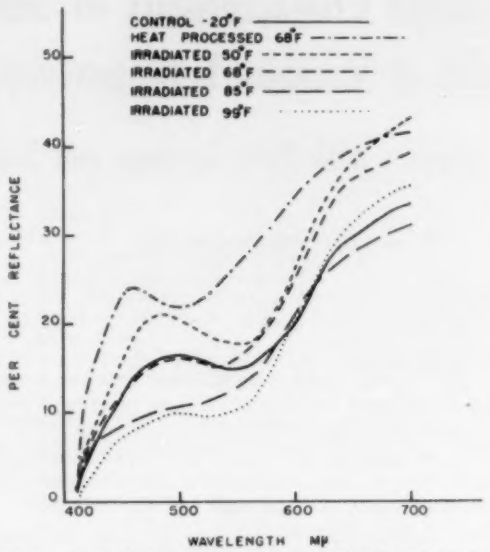

Figure 3. Reflectance curves for irradiated (3.5×10^6 rep) and non-irradiated pork sausage held at various temperatures for eight months.

enzyme reaction rate was usually at a maximum during the first 2 months, and at temperatures of 68° F. or greater, a decrease in free amino nitrogen content was observed during the latter part of storage. It may be possible that the amino acids formed were being depleted by the nonenzymatic browning reaction.

Immediately after irradiation there was hardly any color difference between irradiated and unirradiated sausage patties; both were brownish-pink. Irradiated samples held at 50° F. still had good color after 8 months, whereas samples held at 68° F. had assumed an unnatural pink color, and samples kept at 85° F. were pinkish-orange. Irradiated samples stored at 99° F. or 125° F. had turned reddish-brown after 4 to 6 months. Reflectance curves taken after 8 months' storage are presented in Figure 3.

A slightly rancid flavor was detected by the taste panel in irradiated samples held at the low temperatures after a 2-month storage period and was evident in those samples (held at 50° F. or 68° F.) in all subsequent flavor testing. No rancid flavor was detected among irradiated samples held at temperatures above 68° F.; however, it is possible that the rancid flavor could have been masked by other off-flavors which had developed in samples held at the higher temperatures.

Except for one particular instance (8 months' storage at 50° F.) the peroxide test indicated no fat oxidation was occurring in pork sausage. However, the thiobarbituric acid test (TBA) did seem to indicate that some change in the fat was occurring since the TBA value of samples held at 50° F. or 68° F. was usually higher than the TBA value of samples held at temperatures above 68° F. (Table 1).

Another interesting observation was the increase in TBA value caused by the irradiation treatment.

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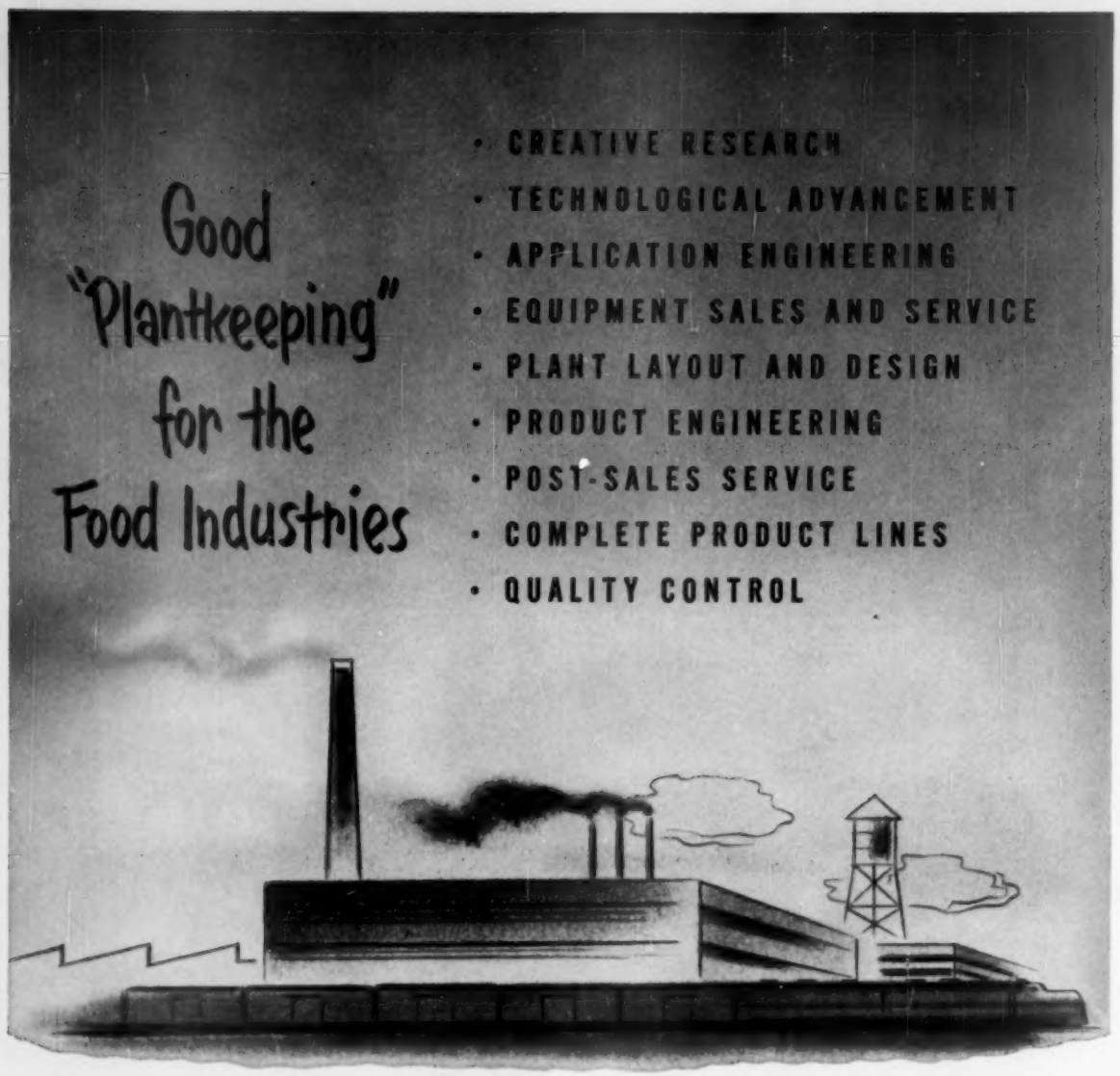

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TABLE 1

Peroxide number and thiobarbituric acid value (TBA) of irradiated (3.5×10^6 rep), non-irradiated and heat-processed pork sausage held at different temperatures

Product	Storage time (months)	Chemical test	−20° F. control	68° F. heat processed	50° F. Irrad.	68° F. Irrad.	85° F. Irrad.	99° F. Irrad.	125° F. Irrad.
Pork sausage	0	Peroxide No.[1]	0	0	0	0	0	0	0
		TBA value [2]	0.183	0.171	0.294	0.294	0.294	0.294	0.294
	2	Peroxide No.	0	0	1	0	0	0	0
		TBA value	0.211	0.170	0.318	0.262	0.265	0.288	0.287
	4	Peroxide No.	0	0	0	0	0	0	0
		TBA value	0.13	0.21	2.74	1.30	0.36	0.40	0.18
	6	Peroxide No.	0	0	0	0	0	0	0
		TBA value	0.56	0.11	0.39	0.47	0.40	0.39	0.35
	8	Peroxide No.	0	0	20	0	0	0	
		TBA value	0.61	0.20	2.24	1.82	0.34	0.49	

[1] = millimoles/kg. fat.
[2] = optical density.

Scallops. The approximate time at which irradiated scallops held at particular storage temperatures became unacceptable in flavor may be obtained from Figure 4. Irradiated scallops held at 50° F. or 68° F. were still acceptable after 10 months of storage. Irradiated samples held at 125° F. became unacceptable in about one month, samples stored at 99° F. were no longer acceptable after approximately 3 months, and samples stored at 85° F. lost their acceptability after 6 months' storage.

Although the irradiated samples were found to be significantly different in flavor from the controls at the start of storage, they were still regarded by all the judges as acceptable. Throughout a storage period of 10 months, a significant flavor difference between frozen controls and irradiated samples held at various temperatures was consistently observed. However, the taste panel response to irradiated scallops held at the lowest storage temperature (50° F.) indicated a high degree of acceptability for this particular sample throughout a 10-month period. Statistical analysis showed the importance of storage temperature for irradiated scallops. For example, the irradiated samples at 50° F. were significantly different from irradiated samples at 68° F., and the latter were significantly different from irradiated samples held at 85° F., and so on, with storage temperatures of 99° F. and 125° F.

Factors which contributed to the unacceptability of the irradiated product during storage included a darkening in color, a softening in texture, and the development of an acidic taste. These deteriorative changes were quite obvious in samples stored at 99° F. or 125° F. after 2 months, and in samples held at 85° F. after 6 months.

Results of free-amino-nitrogen determinations made on irradiated and non-irradiated scallops during 10 months of storage are presented graphically in Figure 5. In general, it appeared that proteolytic enzyme activity was absent during storage; however, a few random points seemed to diverge from the general path and it was believed that these particular samples had not been adequately blanched. It may be possible that a slight amount of proteolysis occurred during the first two months, but it seems more probable that the amino nitrogen content of the irradiated samples at zero storage time was in error and should have been slightly higher. It may be noted that in products in which proteolysis occurred, there was a definite relation between amino nitrogen content and storage temperature, with a greater concentration resulting at the higher temperatures. In the case of scallops, no such definite relationship existed.

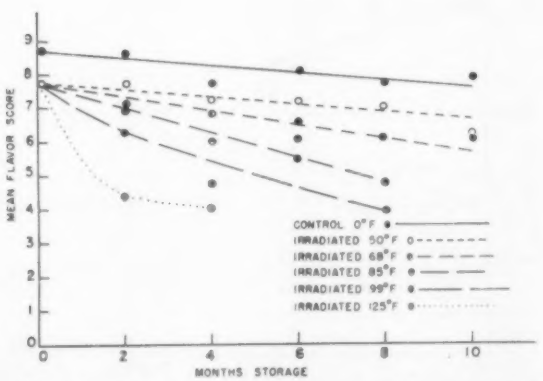

Figure 4. Effect of storage time on mean flavor scores of non-irradiated and irradiated (3×10^6 rep) scallops held at various temperatures.

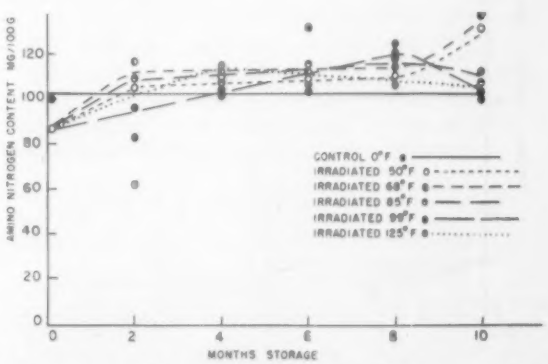

Figure 5. Amino nitrogen content of irradiated (3×10^6 rep) and non-irradiated scallops stored at various temperatures.

Irradiated and non-irradiated scallops were indistinguishable in color at the start of storage tests, but with increasing storage time a darkening of the irradiated samples occurred, probably as a result of non-enzymatic browning. The off-color was a function of the storage temperature and varied from light grey or off-white for samples held at 50° F. to an orange-tan for samples held at 125° F. Scallops held at 125° F. had assumed a deep-tan coloring after 2 months and a transition to orange-tan occurred by the end of 4 months. Samples stored at 99° F. reached this color stage after 6 to 8 months. After 8 months irradiated samples held at 50° F. were still grayish-white and samples stored at 68° F. had become light tan.

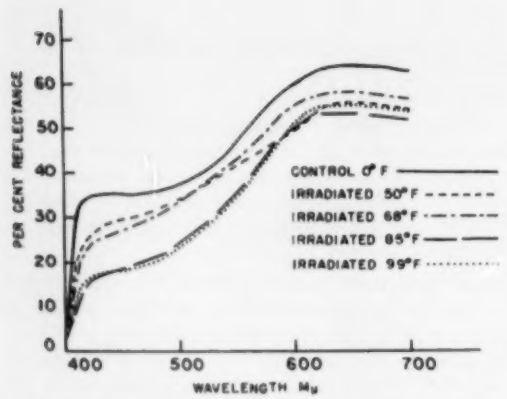

Figure 6. Reflectance curves for irradiated (3×10^5 rep) and non-irradiated scallops held for eight months at various temperatures.

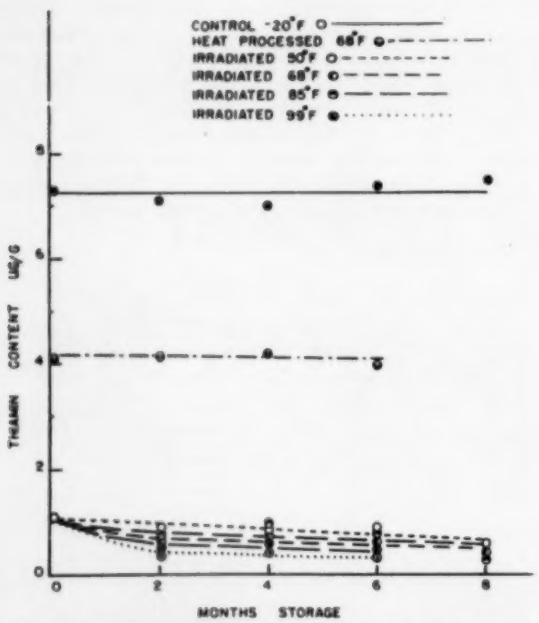

Figure 7. Thiamin content of irradiated (3.5×10^5 rep) and non-irradiated pork sausage held at various temperatures.

Reflectance curves taken after 8 months' storage are shown in Figure 6.

Stability of certain vitamins in irradiated products held at various storage temperatures. The thiamin, riboflavin, and pyridoxine contents of irradiated and non-irradiated pork sausage and scallops are presented in Figures 7 through 12 as a function of storage time at various temperatures.

The irradiation treatment destroyed a significant amount of thiamin (85%) and riboflavin (40%) in pork sausage, whereas loss of pyridoxine only amounted to about 10%. In general, the heat-process

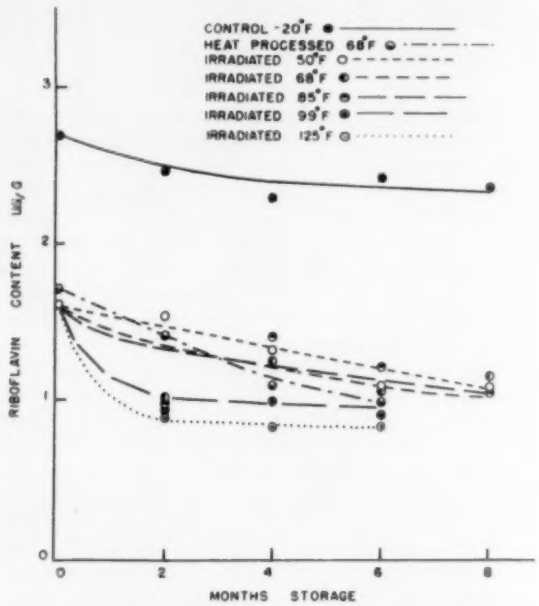

Figure 8. Riboflavin content of irradiated (3.5×10^5 rep) and non-irradiated pork sausage held at various temperatures.

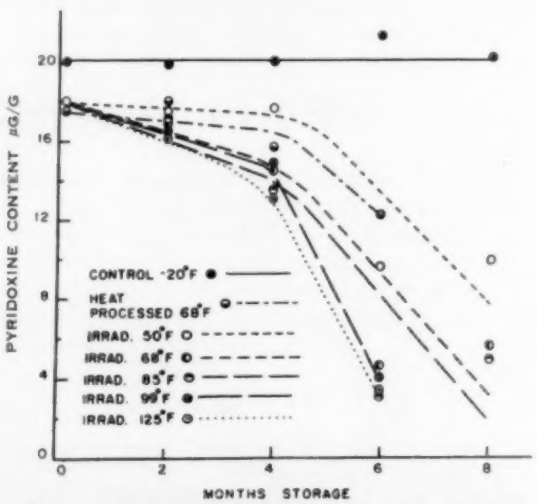

Figure 9. Pyridoxine content of irradiated (3.5×10^5 rep) and non-irradiated pork sausage held at various temperatures.

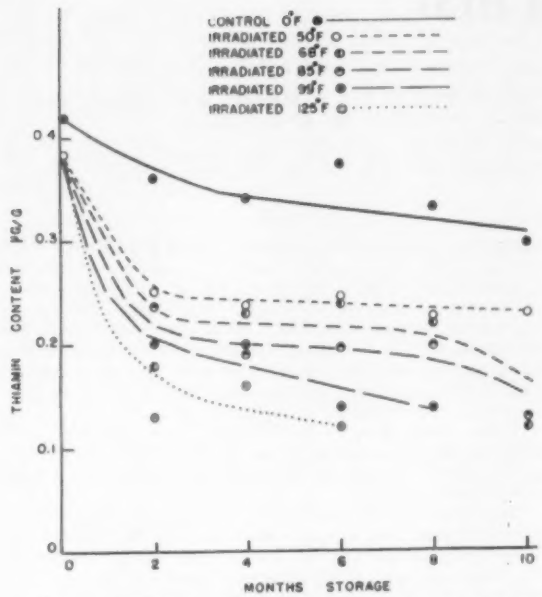

Figure 10. Thiamin content of irradiated (3×10^6 rep) and non-irradiated scallops held at various temperatures.

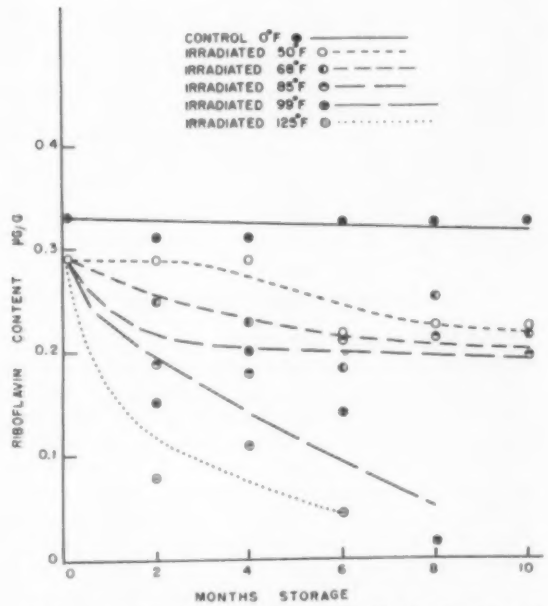

Figure 11. Riboflavin content of irradiated (3×10^6 rep) and non-irradiated scallops held at various temperatures.

treatment caused about the same amount of destruction of these vitamins as did irradiation.

In the case of scallops, irradiation induced only about a 10% reduction of thiamin, riboflavin or pyridoxine contents.

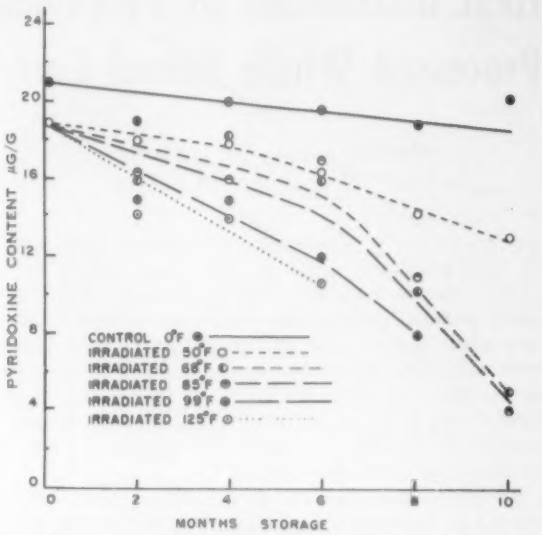

Figure 12. Pyridoxine content of irradiated (3×10^6 rep) and non-irradiated scallops held at various temperatures.

During storage the rate of vitamin destruction among irradiated samples was a function of temperature, with a greater loss resulting as storage temperature increased.

SUMMARY

Storage studies were carried out with irradiated pork sausage and scallops held for eight to ten months at temperatures ranging from 50° F. to 125° F.

Even after eight months' storage at 50° F. irradiated pork sausage was considered acceptable in flavor. At storage temperatures of 68° F. or above, irradiated samples became unacceptable within two months.

Irradiated scallops were considered to be acceptable after 10 months' storage at 50° F. or 68° F.; however, a marked deterioration in quality occurred within one month in samples held at 125° F.

Non-enzymatic browning, which caused off-flavor and color degradation, played the principal role in the deterioration of irradiated scallops during storage, but texture losses also occurred.

In the case of irradiated pork sausage, texture change and associated off-flavors due to proteolytic enzyme action chiefly contributed to loss of quality. Color changes and fat oxidation also occurred.

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Heat Inactivation of Peroxidase in HTST Processed Whole Kernel Corn[a, b]

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(Manuscript received March 1, 1958)

STUDIES HAVE recently been made in this laboratory *(4)* on heat inactivation time (HIT) curves for sweet corn peroxidase. The partially purified enzyme was heated in capillary tubes and whole kernel corn was heated in thermal death time cans. The resulting curves showed high z values as compared to those for bacterial spores indicating that under high temperature-short time processing conditions canned corn might be sterilized without inactivating peroxidase enzyme.

In this earlier work *(4)* it was found that samples tended to regenerate peroxidase activity during storage of heat inactivated samples. This regeneration of activity was dependent on storage temperature, being more pronounced at 100° F. storage than at 40° or 75° F., and was completed within two days.

The HIT curves constructed from data obtained on the inactivation of peroxidase in whole kernel corn heated in TDT cans were approximate and thus only indicative *(4)*. Therefore, it seemed desirable to obtain data which would permit construction of more accurate HIT curves for peroxidase in whole kernel corn. More nearly commercial conditions were simulated in this study by heating the samples as brine packed whole kernel corn in a pilot model end-over-end agitating cooker. A study was also made on the rate of regeneration of peroxidase activity in such heat inactivated samples.

EXPERIMENTAL

Raw material. Whole kernel sweet corn was prepared from freshly harvested Golden Cross sweet corn by mechanically cutting the kernels from the cobs. After washing in a rod reel washer, the kernels were frozen and held at −40° F. until needed. The corn was defrosted overnight at room temperature.

Method of heating. The corn was processed as brine packed whole kernel corn in Number 1 "C" enamel cans. Seven ounces of kernels were weighed into each can. Boiling brine was added and the gross headspace adjusted to 4/16th inch by means of a topper. The brine solution was prepared on the basis of 7 pounds NaCl and 14 pounds sucrose per 100 gallons water. The cans were closed and processed immediately.

The cans were heated in a pilot model end-over-end agitating cooker having a capacity of 20 Number 1 cans. For the determination of the HIT curve for sweet corn peroxidase under these conditions, 2 cans were processed at one time. One can was analyzed immediately and the other was placed in storage to allow regeneration. An additional test can equipped with a thermocouple was included to determine heat penetration. The remainder of the spaces in the retort were filled with cans containing water. At process temperatures of 260° F. and above heating times were in increments of 10 to 15 seconds. At process temperatures of 240° F. and 250° F. heating times were in increments of 20 to 30 seconds. In studies on the rate of regeneration of enzyme activity, all 20 spaces were filled with sample cans. A rotational speed of 80 r.p.m. was used. Timing of the process was from steam on to steam off. At the end of heating the cans were cooled to about 100° F. under air pressure with tap water flowing through the retort.

Process temperatures were 240°, 250°, 260°, 270°, 280°, 290°, and 300° F. in the determination of the HIT curve. Samples used to study the rate of regeneration of peroxidase activity were heated at 260° F.

Heat penetration studies. The agitating cooker was equipped with a slip ring type commutator which facilitated temperature measurement inside the can during processing. Temperature readings were made at the geometric center of the can using Ecklund type thermocouples connected through the commutator to a Minneapolis-Honeywell 12-point recording potentiometer. The thermocouple was connected to all 12 points of the instrument so that each point recorded its temperature, thus producing a smooth heat penetration curve. This curve was then used to correct heating times for the lag in heating and cooling of the contents of the cans. In the determination of the correction factor *(4)*, z values of 42° and 86° F. for temperatures below and above 270° F., respectively, were used.

Storage conditions. Samples were analyzed for peroxidase activity within 30 minutes or after 72 hours storage at 75° F. for the determination of the HIT curves. In the study on the rate of regeneration of peroxidase activity, samples were analyzed for enzyme activity after 0, 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours storage at 75° F.

Enzyme analysis. Analysis for peroxidase activity in the kernels was made by the o-phenylenediamine method of Vetter, Steinberg, and Nelson *(5)*.

RESULTS AND DISCUSSION

Heat inactivation time curves. Figure 1 shows HIT curves for sweet corn peroxidase under the conditions of this experiment. Each pair of points (O and X) represents a single series of samples, each of which was heat processed for a different time at the indicated retort temperature. Each sample of the series was analyzed for residual enzyme activity. As the heating time was increased the activity decreased to the point where it could no longer be detected by the analytical method employed. Longer heating times resulted in negative activity. Only the longest time which left some activity (X) and the shortest time resulting in negative activity (O) are shown plotted. Thus, the actual time that would be required to completely inactivate the enzyme is "bracketed" by this pair of points. The pairs of points in Curve A are based on enzyme analyses made immediately after each sample had been processed. Each pair of points in Curve B is based on a series of samples which was prepared and processed as a duplicate of that used for Curve A at the same temperature, but had been stored for 72 hours at 75° F. in order to allow for any regeneration of enzyme activity.

[a] Submitted in partial fulfillment of the requirements for the Ph.D. degree in Food Technology, University of Illinois, Urbana, Illinois.

[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May 27, 1958.

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If the pairs of points of Curve A are connected by the solid line shown, a z value of 59° F., a F_{250} value of 2.3 minutes and a F_{300} value of 0.49 minutes are indicated. This HIT curve may also be considered as two intersecting straight lines, shown as broken lines. In this case F_{250} = 3.5 minutes, F_{300} = 0.57 minutes and z values are 49° F. and 70° F. at the low and high temperatures, respectively.

The break in Curve B is immediately apparent. Two straight lines were fitted to the data as shown. The low temperature line is characterized by a F_{250} of 7.0 minutes and a z value of 70° F.; the high temperature line by a F_{300} of 2.9 minutes and a z value of over 300° F. This break at about 270° F. and the slopes or z values found here are similar to previously published data for sweet corn peroxidase *(4)* and may be of theoretical significance.

These findings can be explained by the supposition that sweet corn peroxidase is composed of 2 enzymes differing in their heat resistance. Theorell *(3)* separated horseradish peroxidase into two separate enzymes using electrophoresis. Some time later Jermyn *(2)* was able to isolate 4 different enzymes showing peroxidase activity when a horseradish peroxidase preparation was subjected to chromatographic analysis. Peroxidases from different sources were shown to vary in their heat inactivation properties by Esselen and Anderson *(1)*. Thus, at temperatures below 270° F. the heat inactivation reaction could be controlled by one enzyme having a low z value but high F_{250} value while above this inflection point the second enzyme of high z value but low F_{250} value would be the more heat resistant.

HIT curves for enzymes are usually presented as straight lines as is done in the case of thermal death time curves for bacteria. In order for the HIT curve to be a straight line the Q_{10} of the inactivation process would have to remain constant over the temperature range studied. In the usual chemical reaction, the Q_{10} asymptotically approaches one as temperatures are increased. If this is true for heat inactivation of enzymes, the HIT curve would level off and approach a horizontal line at elevated temperatures. Therefore, it may be that data in Figure 1 should be connected by continuous curves instead of by straight lines as shown.

Heat inactivation of peroxidase is so rapid at temperatures above 270° F. that the correction for come-up and cooling times becomes appreciable in relation to the calculated effective heating time. Since this correction is itself an experimentally determined value, its inherent error may be additive to other experimental errors and thus cause aberration in the high temperature portion of the curve. The total aberration for Curve B, or the solid line vs. the broken line extension of the low temperature portion of this curve is only 1.6 minutes at 300° F. Thus, one might be tempted to attribute the break in Curve B to experimental error. However, the data for Curve A were obtained and corrected in exactly the same manner as those for Curve B and it shows what may be a minor break in the curve. This lends support to the conclusion that the break in Curve B is indeed significant.

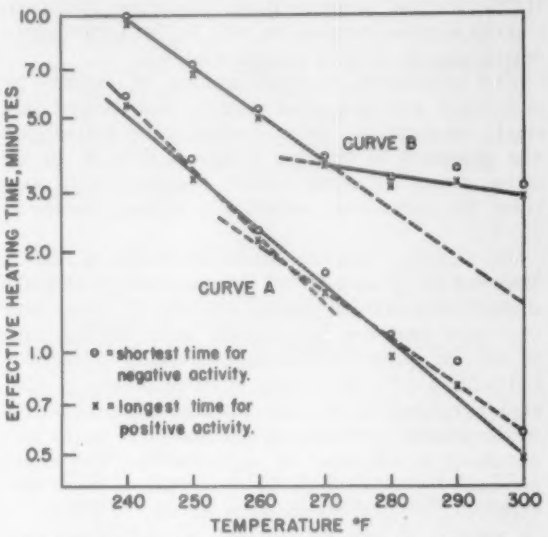


Figure 1. Heat inactivation time curves for peroxidase in whole kernel corn heated in the agitating cooker as determined immediately after heating (A) and after 72 hours storage at 75° F. (B).

Regeneration of peroxidase activity. The regain of some enzymatic activity by a heated vegetable preparation during subsequent storage is known as enzyme regeneration. This was noted in previous work with peroxidase activity of sweet corn extracts *(4)*. In the present work processing temperature and storage time were separately studied for their effect on regeneration of peroxidase in brine packed whole kernel sweet corn.

The data on effect of processing temperature in the end-over-end agitating retort are summarized in Figure 1. Curves A and B were explained at the beginning of this section. The relationship between the curves is of interest here. The time difference between them at any given temperature shows how much longer the product had to be heated at that temperature in order to prevent some regeneration of the enzyme.

Over the temperature range 240° F. to 270° F. the 2 curves of Figure 1 are almost parallel. Since heating time is plotted on a logarithmic scale here, this means the ratio between times A and B at any given temperature is constant. Thus, over this temperature range, approximately twice (2.0 to 2.3) as long a heating time was required to give a permanent peroxidase inactivation as to produce an initial inactivation. The time required to prevent regeneration under these conditions, Curve B, decreased from 9.8 minutes at 240° F. to 3.7 minutes at 270° F.

At temperatures above 270° F. the curves diverge so that at 300° F. almost 6 times as long was required to prevent regeneration, 2.9 minutes, as compared to inactivation immediately after heating. This finding emphasizes the importance of enzyme regeneration in

HTST thermal processing and indicates that permanent enzyme inactivation may be the factor which determines the upper temperature limit.

The relationship between amount of enzyme regeneration and time after heating was studied at a single temperature. Brine packed whole kernel corn was processed in Number 1 cans at 260° F. in the end-over-end agitating cooker. Samples were analyzed for peroxidase periodically during storage at 75° F.

The results of this experiment are shown in Table 1. The first lot, processed for approximately 5 minutes, showed no detectable peroxidase activity when samples were analyzed immediately after heating. The second lot, processed for approximately 4 minutes, indicated a residual activity of 0.022 O.D.U./g. when analyzed immediately after heating. In both lots it was impossible to draw any conclusions as to the time necessary to complete the regeneration. The results obtained for cans stored for more than 7 hours were erratic. These variations could be due to differences in amount of heating received by individual cans.

TABLE 1

Regeneration of peroxidase activity in whole kernel corn processed for four or five minutes at 260° F. in agitating cooker

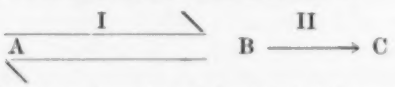
Storage time (hours)	Enzyme concentration (O.D.U./g.)	
	Processed 5 min.	Processed 4 min.
0	.00	.022
	.00	.022
1	.016	.032
	.015	.019
2	.020	.072
	.021	.....
4	.031	.156
	.013	.128
7	.081	.147
	.062	.210
12	.056	.244
	.051	.346
24	.....	.552
	.086	.176
48	.154	.217
	.139	.180
72	.215	.492
	.....	.152
96	.151	.451
	.....	.498

Fill-in weight and headspace affect the rate of heat penetration into cans processed in an agitating cooker. Any variation in these conditions would cause a slight difference in amount of enzyme inactivation. Fill-in weight was controlled at 7 ounces and headspace was adjusted to 4/16th inch. This gross headspace left only a 2/16th inch net headspace after the cans were closed. Therefore, any slight error in adjusting the headspace would be reflected by differences in the rate of heat penetration into the can and in amount of enzyme inactivation. These differences could be minimized by using a small fill-in weight (about 6 ounces) and a larger headspace (about ½ inch).

The enzyme content of samples stored at various times through 7 hours (Table 1) were plotted against time of storage. Straight lines were indicated and a larger amount of regeneration was shown in samples containing 0.022 O.D.U./g. residual peroxidase activity. The slope of the line for samples exhibiting no residual enzyme activity immediately after heating was approximately 0.009 O.D.U./hour. The slope of the line for samples exhibiting 0.022 O.D.U./g. residual enzyme activity was approximately 0.024 O.D.U./hour. This indicates that the rate of regeneration of peroxidase activity is much higher when a slight amount of activity remains after processing.

In both lots of samples regeneration was detectable after one hour of storage. Therefore, if a study is to be made of the effect of heating on the residual enzyme content of processed corn, analysis for this activity should be made as soon as possible after heating. It is recommended that no longer than 30 minutes elapse between processing and analyzing.

The irreversible heat inactivation of peroxidase is probably a two-step reaction illustrated as:



In reaction I an inactive product (B) is formed which is capable of reverting back to the original active state (A). Further heating would then irreversibly convert (B) to product (C) and no regeneration of activity would be possible. Thus, if immediately after heating, a sample shows some enzyme activity, it may be that reaction I is still in progress and a large proportion of enzyme exists as (B). The tendency then would be for a more rapid and increased amount of regeneration. Slightly longer heating could then complete reaction I and inactivation to (C), the irreversible form, would predominate. If the heating was not sufficiently long to *complete* reaction II, then the enzyme in form (B) would tend to regenerate, but at a slower rate. As the time of heating increased, the quantity of product (B) which reverted to (A) and the rate of this reaction would be expected to decrease until they reached zero at the point of complete conversion to (C).

Enzyme molecules are though to exist in a helical configuration with catalytically active sites at critical points on the external surface. Inactivation of enzymes by heat probably is the result of thermal denaturation of protein, which is primarily a change in geometric configuration. When an enzyme molecule is heated, the helical shape is altered and the spatial arrangement of catalytic sites is disturbed. The molecule then loses its ability to form enzyme-substrate complexes and, consequently, its catalytic activity. It seems reasonable to assume, in the case of an enzyme such as peroxidase which exhibits the ability to regain activity after temporary heat inactivation, that relatively mild heating alters the shape of the molecule sufficiently to cause it to lose its catalytic activity but somewhat longer heating is required to change the configuration so drastically that return to original form is prohibited.

SUMMARY

Whole kernel sweet corn was processed under semi-commercial conditions in a pilot model end-over-end agitating cooker. Two heat inactivation time curves were obtained over the temperature range 240° F. to 300° F. The curve based on samples analyzed immediately after heating showed a F_{250} value of 3.43 minutes and a F_{300} value of 0.49 minutes with a z value of 59° F. When samples were allowed to regenerate enzyme activity during 72 hours storage at 75° F., the heat inactivation time curve exhibited a F_{250} value of 7.0 minutes and F_{300} value of 2.9 minutes. A distinct inflection point was found at about 270° F. The z values were 70° and over 300° F. for temperatures below and above 270° F., respectively.

The theoretical significance of the change in slope of heat inactivation time curves was discussed. It was pointed out that this may be the result of more than one enzyme exhibiting peroxidase activity and that the "curve" may actually be curvilinear and not a straight line.

Studies were made on the regeneration of peroxidase activity in heat inactivated samples. It was found that regeneration began immediately after heating and that it was more rapid and extensive in samples containing a small amount of residual enzyme immediately after heating than in samples showing no residual activity at that time. It was recommended that, when studies are to be made on the effect of heat on the residual peroxidase activity of sweet corn, analysis for this activity be made within 30 minutes of heating.

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A Rapid Spectrophotometric Method for the Detection and Estimation of Adulterants in Tomato Ketchup

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TOMATO KETCHUP, according to internationally accepted convention, is defined as *the clean sound product made from properly strained tomatoes with spices, salt, sugar and vinegar, with or without onion and garlic*, and containing not less than 12% of tomato solids *(1)*. Various other fruit and vegetable pulps are often employed, however, as filling materials to increase yields and reduce costs. Among the adulterants employed, carrot, pumpkin, ash gourd, sweet potato, papaya and apple are the more important ones in India. Any defect by way of reduction in the colour of the ketchup is often made up by adding bright red food colours. This is an undesirable state of affairs and in the best interests of the fast-developing tomato products industry in this country should be stopped. The detection and estimation of this type of adulteration with cheaper materials is no doubt an important problem elsewhere, and a systematic study will therefore be useful.

The problem of adulteration in tomato products has been the subject of several investigations in recent years. Morpeth *(11)* reported that potash content, combined acids and total acids were of special significance for determining tomato solids in ketchups made from puree. Bigelow, Smith and Greenleaf *(2)* proposed two methods, one based on non-sugar solids and the other based on insoluble solids. Darbishire *(6)* made use of a Lovibond tintometer and a light petroleum ether extract of tomato puree to determine tomato solids. Stock *(13)* suggested an improved technique for correlating the lycopene content with the tomato solids of a sample. Kramer *(9)* has made use of the difference in lycopene contents of drained tomatoes and tomato juice for the detection of water added to the covering juice. Sanahuja *(12)* has evolved a relationship between lycopene, β-carotene and total carotene to detect the use of green tomatoes and carrots in tomato extracts.

In other techniques applicable in the case of other fruit products, chromatographic characteristics, microscopic features and staining characteristics have been utilized *(2, 5, 8, 10)*.

In the present investigation, it was felt that a detailed investigation based on lycopene, which is a distinguishing and characteristic carotenoid pigment naturally present in ripe tomatoes, would lead to results that could form the basis for a distinctive analytical technique for the determination of tomato solids in tomato ketchup and similar products and thereby make it possible to estimate the extent of adulteration with other fruit and vegetable pulps.

The results of this study, with particular reference to lycopene as an index for measuring the purity of tomato ketchup, are presented in this paper. Detailed results regarding other analytical constituents like crude fibre, sugars, acids, polyphenols and histological and staining characteristics will be presented in subsequent papers.

EXPERIMENTAL

Raw Materials

Tomatoes. Fruit of good quality were obtained from the market. The juice was extracted by the hot-break method using a pulper with perforated sieve to separate the juice from the skin and seeds.

Adulterants

Pumpkin and ash gourd were peeled and the inner fibrous portion and seeds discarded. Carrots were scraped. Sweet potatoes were peeled and kept in water to avoid browning prior to subsequent preparation. Papaya was peeled and seeds discarded. Apples were peeled and cored. The prepared material was then cut into small pieces, softened by boiling with a small quantity of water and rubbed through a 30-mesh stainless-steel sieve to get the pulp. Ash gourd and carrots were softened by cooking for about 30 minutes at 10 lb. pressure. The weight of the material before and after softening was recorded in order to know the weight of pulp to be taken for the preparation of adulterated samples of ketchup. For this, the different fruits and vegetables were analysed previously for the total as well as soluble solids contents.

Tomato Ketchup

Ketchup was prepared using the following recipe and boiled down to a total soluble content of 28–30%. The finished ketchup was packed in 8 oz. crown cap bottles and stored for further observations and analysis.

1. Tomato juice	100	oz.
2. Sugar	6	oz.
3. Salt	1	oz.
4. Onion	1.25	oz.
5. Garlic	3	g.
6. Clove	1.5	g.
7. Cinnamon	1.5	g.
8. Black Pepper	0.5	g.
9. Cardamom	0.5	g.
10. Cumin	0.5	g.
11. Mace	0.1	g.
12. Red Chilli	0.6	g.
13. Acetic Acid	14	ml.
14. Sodium Benzoate	1	g.

For the sake of uniformity, the different experimental lots of ketchup, genuine as well as adulterated, were prepared from the same batch of tomato juice. The replacement of tomato solids in the recipe by that of the adulterant was to the extent of 25%, as this was thought to be the level at which different adulterants were likely to be employed in the industry.

Estimation of Lycopene and β-Carotene in Raw Material and Ketchups

A weighed amount of the material, 5–20 g., depending upon its carotenoid pigments content, was extracted with 50–80 ml. of acetone in 4–5 lots, and from the acetone extract the carotenoids were extracted with petroleum ether (BP. 60–80° C.). The petroleum ether extract was made up to volume and kept in an amber-colored bottle for the subsequent estimations.

β-Carotene

From an aliquot of the petroleum ether stock solution β-carotene was separated on a magnesia-super cel (1:3) column and eluted with 3% acetone in petroleum ether, according to the procedure given in *Methods of Vitamin Assay (1)*. The absorbance of the eluent was measured on a DU Model Beckman Spectrophotometer at 452 mμ and also at 473 mμ and 503 mμ. The molecular extinction coefficent ($E^{mol.1cm.}$) of 13.9 x 10^4 for all-trans β-carotene at 452 mμ, given by Zechmeister and Polgar *(14)*, was used to calculate the β-carotene content of the extract.

Lycopene

The stock solution of petroleum ether extract was suitably diluted and its absorbance measured at 473 mμ and 503 mμ. According to Zechmeister *et al. (15)* the molecular extinction coefficient for all-trans lycopene at 473 mμ is 18.6 x 10^4 and at 503 mμ, 17.2 x 10^4, and these values were adopted for calculation of lycopene content in two ways.

Method I. Since β-carotene has a considerable absorbance at 473 mμ, which is the optimum peak for lycopene, correction has to be made for this. From the absorbance reading at 473 mμ for the total carotenoid pigments, the absorbance due to β-carotene at 473 mμ, which was taken on the chromatographically separated β-carotene, was deducted and the lycopene content deduced. This is the accurate method.

Method II. At 503 mμ, β-carotene has a comparatively negligible absorbance, while lycopene has a large absorbance. It is, therefore, not quite essential to apply correction for the the presence of β-carotene in the extract for total carotenoids, especially when the proportion of β-carotene to lycopene is small, as in the case of tomato products. From the direct measurement at 503 mμ, using the total carotenoids extract itself, the lycopene content could be calculated. This is the more rapid method suitable for routine control work as it avoids the chromatographic separation of the β-carotene.

Measuring Lycopene and β-Carotene in Mixtures of Pure Components

To study the validity of the presumption made in the application of the accurate and rapid methods described above, pure lycopene was prepared from tomato paste according to the method of Davis *(7)* and pure β-carotene from carrots according to the *Methods of Vitamin Assay (1)*. These two were mixed in a wide range of proportions (1:1.7 to 1:140) after standardising spectrophotometrically and lycopene values obtained by the two methods compared with the theoretical values. The errors involved were quite small (2–5% only).

RESULTS AND DISCUSSION

Composition of raw materials. Data regarding the total solids, soluble solids as measured on a refractometer at 20° C. using the press juice of the material, β-carotene and lycopene contents of tomatoes and the different adulterants studied are given in Table 1. It will be noticed that the lycopene content of all the other materials except tomatoes is quite small. In the case of carrots, the β-carotene content is very high (78.9 μg./g.). Green tomatoes, which are quite mature, but have not yet developed any red colour, contain very little β-carotene as well as lycopene (1.5 and 1.7 μg./g. respectively). Partially-ripe, yellowish-red tomatoes contain appreciably more lycopene (24 μg./g.), but the fully-ripe red tomatoes contain the maximum amount of lycopene (37.1 μg./g.).

TABLE 1

β-carotene and lycopene content of tomatoes and adulterants (Results on fresh weight basis)

Material	Soluble solids	Total solids	β-carotene	Lycopene
	%	%	μg./g.	μg./g.
1. Tomato juice (ripe)	5.8	6.3	6.1	37.1
2. Carrot	10.0	12.0	78.9	6.5
3. Ash gourd	3.0	4.3	Traces	Traces
4. Pumpkin	6.0	7.7	7.6	3.8
5. Sweet potato	20.0	28.0	.....	0.2
6. Papaya	8.0	9.5	7.1	1.1
7. Apple pulp	10.5	11.8	0.5	1.8
a. Juice from green tomatoes	5.8	.....	1.5	1.71
b. Juice from partially ripe, yellowish-red tomatoes	5.7	.....	5.5	24.0

Estimation of lycopene in mixtures of lycopene and β-carotene. The absorbance of the different mixtures of lycopene and β-carotene in solution was measured at 473 mμ and 503 mμ and the concentration of lycopene calculated at the respective wave-lengths with and without correction for interference by the presence of β-carotene. The difference between the actual and estimated values has been expressed as a percentage of the actual value to know the extent of variation in the wide range studied. The data are shown in Table 2. It will be seen that in the case of the uncorrected values for lycopene at 473 mμ the difference between the actual and estimated values increases with the increase in the proportion of β-carotene. When due correction is, however, made for the interference of β-carotene, the differences become quite small. At 503 mμ also, the differences are quite small. Further, the estimated values agree closely with the actual values over a very wide range of lycopene to carotene ratio. In the range of 1:2.8 to 1:10.4, which is of interest in the case of tomato products, the error likely to be introduced as a result of measuring lycopene directly at 503 mμ in mixed solution is of the order of 3–5% only. The errors are, however, slightly less (2.5–3.5%) when the absorbance is measured at 473 mμ and corrections made. In spite of this, measurement of absorbance of the total carotenoid extract at 503 mμ is to be preferred on account of its simplicity and rapidity for routine quality control work.

Effect of adulteration on the lycopene and β-carotene content of tomato ketchup. The lycopene and β-carotene content of genuine and adulterated samples of tomato ketchup prepared from the May crop are shown in Table 3. It will be seen that in the case of ketchup containing added carrot, the carotene content is high (28.4 μg./g.). As a result of addition of the different adulterants the lycopene content has been reduced in all cases from 69.3 μg./g. to 46.0–50.7 μg./g. of the ketchup. The high carotene value reveals the presence of a carotene-rich material in the ketchup, and in this case it is carrot pulp.

Determination of tomato solids in different tomato ketchups based on lycopene content. Since the lyco-

TABLE 2

Estimation of lycopene in mixtures of pure lycopene and β-carotene

Carotene lycopene ratio[1]	Lycopene taken	At 473 mμ				At 503 mμ			
		Not corrected		Corrected		Not corrected		Corrected	
		Lycopene found	Difference	Lycopene found	Difference	Lycopene found	Difference	Lycopene found	Difference
	μg./ml.	μg./ml.	%	μg./ml.	%	μg./ml.	%	μg./ml.	%
Lycopene only	5.76	5.76	.....	.....	.....	5.51	4.3	5.51	4.3
1:140	5.18	5.05	2.5	5.03	2.9	4.90	5.4	4.80	7.4
1:62	4.60	4.63	0.7	4.58	0.4	4.42	4.0	4.42	4.0
1:36	4.03	4.21	4.4	4.41	2.8	3.99	1.0	3.97	1.4
1:15.5	2.88	2.99	3.8	2.80	2.8	2.73	4.9	2.66	7.8
1:10.4	2.30	2.42	5.3	2.36	2.6	2.21	3.8	2.18	5.1
1:6.7	1.72	1.86	6.6	1.67	2.9	1.64	4.7	1.60	6.8
1:3.8	1.15	1.30	12.9	1.11	3.5	1.12	3.3	1.07	7.0
1:1.7	0.58	0.77	33.8	0.56	3.4	0.60	3.4	0.54	6.9

[1] Lycopene solution (5.76 μg./ml.) and β-carotene solution (0.37 μg./ml.) were mixed in different proportions keeping the total volume at 10 ml.

TABLE 3

Effect of adulteration on the lycopene and β-carotene content of tomato ketchup[1]

Sl. No.	Product	Refractometer solids %	Total solids %	Tomato solids %	β-carotene content μg./g. of sample	Lycopene content (at 473 mμ) μg./g. of sample
1	Tomato juice from ripe fruit.....	5.4	5.8	5.8	6.1	37.1
2	Tomato ketchup—genuine.....	29.5	30.4	13.69	12.6	69.3
3	Tomato ketchup with carrot.....	29.0	30.8	10.06	28.4	47.5
4	Tomato ketchup with ash gourd..	28.5	30.0	9.91	9.2	46.0
5	Tomato ketchup with pumpkin....	30.0	31.5	10.43	11.3	50.7
6	Tomato ketchup with papaya.....	29.5	31.0	10.09	11.4	48.1
7	Tomato ketchup with apple pomace.....	29.5	31.2	10.26	11.7	47.9

[1] Tomatoes from May crop used.

pene content of the ketchup depends upon the amount of ripe tomato solids present in it, the lycopene value of the product could be utilized to arrive at the tomato solids content in it. To verify this, the tomato solids content of a series of experimental ketchups was calculated from their lycopene values (Table 4). It will be seen that the values for the tomato solids taken and found agree very closely, indicating thereby the validity of the above assumption.

Variation in the lycopene content of tomato juice and ketchups. Data regarding the lycopene content in ketchups prepared from tomatoes of different stages of maturity and also from two seasonal crops, namely, February and May crops, are shown in Table 5. It will be seen that the lycopene content of ketchups prepared from tomatoes containing appreciable amounts of green tomatoes will be low. The difference between the February and May crops is small (about 7%).

TABLE 4

Tomato solids in tomato ketchup based on lycopene content[1]

Sl. No.	Product	Actual tomato solids	Lycopene content	Tomato solids deduced
		g./100 g.	μg./100 g.	g./100 g.
1	Tomato ketchup, genuine	15.00	7023	.....
2	Tomato ketchup with carrot	11.25	5072	10.83
3	Tomato ketchup with ash gourd	10.31	4790	10.24
4	Tomato ketchup with pumpkin	11.06	5130	10.96
5	Tomato ketchup with sweet potato	11.44	5051	10.79
6	Tomato ketchup with papaya	10.69	5189	11.09
7	Tomato ketchup with apple pulp	11.06	5116	10.93

[1] One gram tomato solids = 7023/15 = 468 μg. of lycopene.

TABLE 5

Variation in the lycopene content of tomato juice and ketchup

Item No.	Product	Tomato solids	Lycopene per gram of tomato solids
		g./100 g.	μg.
1	Juice from mature, but green tomatoes.....	5.8	29
2	Juice from partially-ripe, yellowish-red tomatoes.....	5.7	421
3	Juice from fully-ripe, red tomatoes (May crop).....	5.8	640
4	Tomato ketchup made from item No. 3 juice.....	13.69	506
5	Juice from fully-ripe, red tomatoes (February crop).....	6.5	596
6	Ketchups prepared from item No. 5 juice:		
	i. Tomato ketchup, genuine.....	15.00	468
	ii. Tomato ketchup with carrot.....	11.25	451
	iii. Tomato ketchup with ash gourd.....	10.31	465
	iv. Tomato ketchup with pumpkin.....	11.06	464
	v. Tomato ketchup with sweet potato.....	11.44	421
	vi. Tomato ketchup with papaya.....	10.69	485
	vii. Tomato ketchup with apple pulp.....	11.06	463

Loss of lycopene and β-carotene during the preparation of ketchup. Data regarding losses in lycopene and β-carotene in the preparation of the ketchups are given in Table 6. The comparatively high loss of the

TABLE 6

Loss of lycopene during the preparation of ketchup[1]

Item No.	Product	Lycopene taken	Lycopene found	Loss
		μg./g.	μg./g.	%
1	Ketchup, genuine.....	87.7	70.2	19.9
2	Ketchup with carrot.....	65.8	50.7	22.8
3	Ketchup with ash gourd.....	55.9	47.9	20.0
4	Ketchup with pumpkin.....	64.7	51.3	20.7
5	Ketchup with sweet potato.....	66.9	50.5	24.5
6	Ketchup with papaya.....	63.5	51.9	18.5
7	Ketchup with apple pulp.....	64.7	51.2	20.8

[1] Lycopene content of tomato juice 38 μg./g. and β-carotene content 6 μg./g.

pigments may be partly due to their destruction during the boiling of the materials and partly due to the fact that the value for $E^{1\%}_{1cm.}$ used for the calculation of lycopene content is the one for the all-trans form and not for the thermally-isomerised form. Zechmeister and Polgar *(14)* and Zechmeister *et al. (15)* have shown that for the thermally-isomerised pigments, there is a decrease of 13.4% and 5.8% in the molecular extinction coefficient for lycopene and β-carotene respectively. As a consequence of this, the values deduced for the lycopene and β-carotene contents of the ketchups are apparently somewhat less than the actual values that would have been got by making use of the reduced values for the molecular extinction coefficient.

Effect of storage on the carotenoid contents of tomato ketchup. Data regarding the changes in β-carotene and lycopene contents of a set of ketchup samples stored at 37° C. for a period of 2 months are shown in Table 7. During this comparatively short period of storage at an elevated temperature, there is very little loss in the two constituents. There is actually a small increase in the values, especially in the case of lycopene. A similar increase has been reported by Davis *(7)* in the case of lycopene content of tomato paste after storage. This increase may be due partly to the better extraction of the pigments from the stored material and partly to the slightly decreased interference of β-carotene as a result of isomerisation. Further work is necessary to confirm these findings over a longer period of storage.

Lycopene index for tomato ketchup. Taking into consideration the effect of the various factors studied so far on the lycopene content of tomato ketchup, for all practical purposes, a value of 475 μg. of lycopene per gram of tomato solids may be proposed tentatively as the 'lycopene index', on the presumption that normally ripe red tomatoes are used in the preparation of the ketchup. Any ketchup sample showing a value much less than 15% for tomato solids may be considered as having been adulterated with other fruit or vegetable pulp or as having been prepared from tomatoes that were not fully ripe and contained varying amounts of green tomatoes. The latter is not a serious problem since the use of green tomatoes is generally avoided by the manufacturer as the green colour makes the product brown and requires the addition of extra red colour to mask it, both of which are undesirable.

The tomato solids contents of some commercial samples of tomato ketchups as calculated from their lycopene content were 15.2–21.1%. They appeared to be normal ketchups. More samples from different parts of the country have to be analysed to test the applicability of the lycopene index for detection of adulteration. Work is in progress in this direction. In spite of its limitations, the use of the lycopene value as an index of the 'purity' of tomato ketchup, is a highly useful method for the detection and estimation of adulteration as it is based on a highly characteristic constituent of tomatoes.

SUMMARY

A rapid method has been evolved for the estimation of lycopene in tomato products based on the measurement of absorption at 503 mμ using the petroleum ether extract of the total carotenoids. The replacement of tomato solids in tomato ketchup by such adulterants as carrot, ash gourd, pumpkin, sweet potato, papaya and apple pulps lowers the lycopene content of the product. On this basis the tomato solids content of the product has been determined by estimating its lycopene content.

Adulterants like carrot pulp increase considerably the β-carotene content of the ketchup. There is very little loss in the lycopene content of tomato ketchups, genuine as well as adulterated, during a storage period of 2 months at 37° C. A lycopene index of 475 μg./g. of tomato solids has been tentatively proposed for the estimation of the extent of adulteration in tomato ketchup, when other fruit and vegetable pulps are employed as adulterants.

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TABLE 7

Effect of storage on the carotenoid content of ketchup (2 months at 37° C.)

Values expressed as μg./g. of sample

Item No.	Product	Total solids	Tomato solids	β-carotene		Lycopene	
				Initial	Final	Initial	Final
		%	%				
1	Tomato ketchup—genuine	31.3	15.0	12.15	11.48	70.23	77.66
2	Tomato ketchup with carrots	32.0	11.3	33.56	36.46	50.72	58.48
3	Tomato ketchup with sweet potato	32.0	11.4	9.57	9.00	50.51	54.76
4	Tomato ketchup with pumpkin	31.2	11.1	11.57	10.92	51.30	55.91

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Notes and Letters

The pH Problem in Canned Tomatoes[a, b]

The incidence of high pH values in canned tomatoes in 1957 and 1958 has been of great concern to the canning industry. To quote Dr. Charles Townsend of the National Canners Association: "In the past 3 years we have found a progressive increase in the amount of spoilage. In the laboratory we have found it very difficult to get germination of spores of the butyric anaerobes below about pH 4.5. However, some germination below this point must be possible, based on the spoilage results which we have encountered in the field. The thermophilic anaerobes will germinate down to about pH 4.5 to 4.6" *(1)*.

This value of pH 4.5 is the classical division between acid and non-acid products. Recent studies in commercial canneries have shown pH values as high as 5.0 with reports from Colorado of readings as high as 5.6. If the rise in pH continues, there could be an increased incidence of spoilage and even the occurrence of a public health problem *(1)*.

Higher temperatures and longer processing times could be used to assure the destruction of spores in tomatoes. When tomatoes of pH 3.9 are processed at 212° F., only 34 minutes are required to kill a normal or high spore load, whereas at pH 4.8, the cook must be 110 minutes *(1)*. However, prolonged exposure to elevated temperatures would decrease quality factors of color, flavor, texture, wholeness and ascorbic acid content.

Another alternative, which is discussed in the present paper, is to acidify canned tomatoes to a desirable pH range. This is only one aspect of the problem and while other considerations are under investigation, the indication of this year's experimental work in the use of added acid to lower the pH and added sugar to balance the flavor merit reporting.

PROCEDURES

Midseason tomatoes of the Pearson variety were used. All fruit was selected from field run, well-colored, sound tomatoes. These selected tomatoes were lye peeled, washed and cored. Twenty oz. of tomatoes and 9 oz. of juice were packed into 401 x 411 cans. Juice was prepared from the same tomato sample. Additions of sodium chloride, citric acid and sucrose were made to the juice as indicated in Table 1. After vacuum closing, the canned tomatoes were cooked in an Anderson-Barngrover rotary cooker for 32 min. at 212° F. and cooled. A period of 6 weeks was allowed to permit the fruit and the solids added in juice to reach equilibrium.

Flavor quality evaluations were obtained from 10 highly trained, laboratory panel members who ranked and scored the samples. A balanced, incomplete block design, with 3 samples per taste session, was used. The tomatoes were ranked 1st, 2nd, and 3rd in acceptability as well as being scored on an 8-point scale with "1" denoting poor quality and "8" indicating excellent flavor quality. A total of 60 evaluations per sample was obtained. Aliquots from samples used for flavor evaluation were used for chemical analysis. The flavor data were subjected to analysis of variance for determination of effect of treatment on average scores and average ranks.

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[a] Paper presented at the annual meeting of the Northern California Section of IFT, December 4, 1958.

[b] Supported in part by a grant-in-aid from the Sugar Research Foundation, New York.

TABLE 1

Quality evaluation of canned tomatoes

Code	Additions			Chemical analysis[1]				Taste panel[4, 5]	
	NaCl	Citric-acid	Sucrose	pH	Soluble solids[2]	Total acidity[3]	Brix-Acid ratio	Score	Rank
	%	%	%		°Brix	%			
A	0.5	0.0	0.0	4.45	6.34	0.368	17.3	4.38	2.05
B	0.5	0.1	0.0	4.31	6.55	0.476	13.8	4.17	2.10
C	0.5	0.0	1.0	4.47	7.67	0.376	20.4	3.97	2.00
D	0.5	0.1	1.0	4.28	7.58	0.481	15.9	4.02	2.10
E	1.0	0.0	0.0	4.40	7.18	.380	19.0	3.72	2.23
F	1.0	0.1	0.0	4.28	7.08	0.460	15.4	3.47	2.37
G	1.0	0.0	1.0	4.41	7.79	0.368	21.3	4.67	1.88
H	1.0	0.1	1.0	4.22	7.86	0.465	17.1	4.48	1.72
I	1.0	0.0	2.0	4.42	8.69	0.370	23.5	4.43	2.03
J	1.0	0.1	2.0	4.27	8.86	0.457	19.4	4.73	1.70
K	1.0	0.2	2.0	4.13	8.85	0.560	15.8	4.27	1.92
L	1.0	0.2	3.0	4.11	9.57	0.547	17.5	4.23	1.98
M	1.0	0.3	3.0	4.00	9.72	0.621	15.7	4.45	1.92

[1] Average of 6 determinations.
[2] Soluble solids of pulped tomatoes and juice by refractometry.
[3] Calculated as % citric anhydride.
[4] Average of 60 separate evaluations per sample.
[5] Effect of treatment on average scores and ranks significant at the 0.1% level of probability.

RESULTS AND DISCUSSION

Data obtained on the quality evaluation of acidified and sweetened canned tomatoes are shown in Table 1. Average pH values ranged from 4.47 to 4.00 as a result of acidification.

Table 2 illustrates the range of pH and total acidity on an individual can basis. The analysis of individual cans appears to be highly variable. It is essential to consider the high values in the range rather than the average pH. Acid additions should be sufficient to lower the pH range to a level which will permit minimum sterilization time in boiling water. Sample M, with a pH range of 3.90–4.08 and a total acidity from 0.594–0.635, could be considered well within the levels of safety and had a high flavor acceptability as well. It is important to note that this sample was acidified to doube the total acidity of the control sample (A).

TABLE 2

Range of pH values and total acidity content

Tomato code	No. cans sampled	pH range	Total acidity range
			%
A	18	4.40–4.50	0.349–0.382
B	16	4.22–4.37	0.459–0.497
C	16	4.40–4.55	0.361–0.399
D	14	4.20–4.32	0.452–0.583
E	15	4.32–4.43	0.364–0.394
F	11	4.21–4.32	0.444–0.484
G	12	4.38–4.43	0.323–0.402
H	11	4.20–4.30	0.419–0.505
I	10	4.35–4.47	0.361–0.389
J	10	4.22–4.32	0.447–0.464
K	12	4.08–4.20	0.547–0.577
L	13	4.07–4.20	0.515–0.566
M	11	3.90–4.08	0.594–0.635

Taste panel results indicated that small additions of sucrose were necessary to off-set the sourness contributed by this increased acidity. This is evidenced by the fact that the best liked samples were J, H and G. Among the least acceptable were samples B, D, E and F, the remaining samples receiving intermediate acceptance. Additions of acid and sugar resulted in canned tomatoes which were liked as well as and frequently better than the control (Sample A). It is evident from these data, that careful consideration must be given to the proper balance between saltiness, sourness and sweetness in this product in order to achieve reduced sterilization time and a palatable flavor blend.

CONCLUSION

Acidification of canned tomatoes with citric acid has been suggested as a means of increasing acidity to a safer and more desirable level. Because additions of acid (0.1, 0.2 and 0.3%) increased the sourness of the product, additions of sucrose were needed for compensation. The data point out the variability of total acids, and pH of individual samples.

A considerable amount of additional research on the pH problem is sorely needed. Specifically, further information is needed on microbiological changes, sterilization requirements, processing procedures, drained weight considerations, storage stability and finally, consumer acceptance.

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The Antifungal Antibiotic A-5283, A-5288, Myprozine, Pimaricin

We note in reading an article on the effect of several antifungal antibiotics on the growth of fungi causing food spoilage that Klis *et al. (4)* refer to a paper in which we reported that A-5288 (Myprozine) and Rimocidin effectively inhibited the development of yeast flora. They state that "Tarr *et al. (7)* found rimocidin useful in preventing yeast growth on fish treated with chlortetracycline and Ayres *et al. (3)* reported that rimocidin was effective in inhibiting the development of yeast indigenous to dressed poultry," but that "there was no published information available concerning the properties or activity of myprozine."

We are certain that this oversight would not have occurred had a single code number been used for Myprozine. At the time that we were working with the compound and when the only means of identifying the material was by code number, our original manuscript indicated the antifungal preparation to be "A-5283 (Lederle)." However, we were advised by the manufacturer that its number should be changed to "A-5288" and so we revised the last numeral. Sometime after our paper had been published, we were informed that A-5283, and not A-5288, was the correct code number.

In a later paper, and after A-5283 (or A-5288) had been given the name Myprozine, Ayres and Denisen *(2)* referred to its use as an antifungal agent for maintaining freshness of berries.

In 1958 the Dutch workers, Struyk *et al. (6)* described the isolation and properties of a new crystalline tetraene antifungal antibiotic which they named Pimaricin. Patrick *et al. (5)* reported that a comparison of the infrared and ultraviolet spectra, paper chromatographic mobilities, N-acetyl derivatives, and X-ray powder diffraction patterns of Pimaricin and Myprozine indicated the two compounds to be identical. Recently the Royal Dutch Fermentation Industries at Delft *(1)* has undertaken mass production of Pimaricin.

We hope that the above information will clarify the matter of aliases for Pimaricin. Regardless of whether it is called A-5283, A-5288, Myprozine or Pimaricin, this antifungal antibiotic seems to be effective in inhibiting growth of various yeasts and molds on poultry and fruit.

John C. Ayres
Food Processing Laboratory
Iowa State University
Ames, Iowa

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(Continued from page 24 preceding technical section)

REGIONAL NEWS

CHICAGO

Fall Outing and Tour

The "future meeting date" that the Chicago Section is looking forward to is the September 26, 1959, meeting in Madison, Wisconsin. This event, the traditional opening of the fall schedule of meetings, combines a tour of the University of Wisconsin Dairy and Food Industry Department and other technological features of the institution with a football game; namely, the Wisconsin-Stanford game. Food technologists new in the Chicago area as well as those long established in the area but not yet "joined up" should get in touch with L. B. Buettner, membership chairman for the Chicago Section, International Minerals and Chemicals, Skokie, Illinois.

House Organ Publications

It will be appreciated if members whose companies have house organs that would be receptive to Chicago Section news items would send a copy of the publication to Mr. Norbert J. Leinin, *Food Processing*, 111 E. Delaware Place, Chicago 11.

MOHAWK VALLEY

The Mohawk Valley Section IFT met late in April at the Manor, St. Johnsville, N. Y. Vice-Chairman, John Stewart of the New York State University at Morrisville, introduced Mr. G. T. Peterson, Research and Development Division of Continental Can Co., who spoke on the subject of Foods, Packaging and the Future. Mr. Peterson pointed out that every 7½ seconds a baby is born; that the excess of births over deaths amounts to a full scale city of better than 50,000 population every week of the year; and that based on projected estimates, the United States alone will have 44 million more people to feed by 1975.

Mr. G. T. Peterson

He then spoke of the packaging industry and its role in bringing better foods to the consumer at lower cost, and increased variety and availability. He reviewed the basic characteristics of the different kinds of packaging materials, the function of packaging and its importance in today's economy and stressed the reason proper and tailor-made packaging is so necessary for the successful marketing of any food product.

He pointed out that the science of chemistry is our greatest ally in these developments.

Mr. Peterson concluded by saying "It is not boasting to claim that packaged foods generally have shown

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the least effects of inflation and gradually increasing costs as compared to most things that surround us today. This may be hard to realize since food comprises such a large portion of our budget. However, the food scientist and engineer can justifiably be proud of their contributions to the health and welfare of the world, for without proper food in abundance and availability, none of us would survive."

A lively question and answer period followed Mr. Peterson's talk with Hans Wagner and A. Spaulding, also of Continental Can joining in.

Dorothy Van Schaick

MARYLAND

M.I.F.T.S. held their monthly meeting on May 8, 1959, at Hanover, Pennsylvania, with Hanover Canning Company acting as host.

Starting at 5 o'clock P.M. plant tours were conducted. Visitors witnessed the operation of a high speed Pork N' Bean line which featured dry blanch method of canning plus the automatic can handling and the use of empty can depalitizing systems.

Activities were then directed to the Altland House where Hanover Canning was the sponsor of a cocktail hour after which Mr. Luther K. Bowman, General Manager of the Bowman Agency, presented a most interesting discussion on "The Role of A Management Consultant in the Food Industry." Mr. Bowman explained that the industry has but three really important considerations: (1) People with the know-how and willingness to work, (2) The products or services of their minds and hands in quality and quantity, and (3) Customers. If management is without a full concept of any one or more of these considerations, a management consultant is needed on a part or full time basis.

The services to management were outlined, along with how to select the consultant and working agreements as a part of the company management team.

Fifty-six members and guests were present.

—Warren E. Anderson

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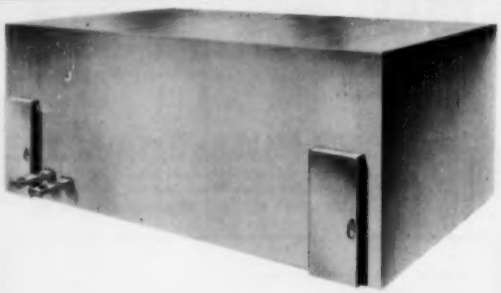

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WASHINGTON

The Washington Section held its regular monthly meeting in the N.C.A. building on May 8, 1959. Nutrition was the subject of the evening. Talks were given by Dr. O. L. Kline, Director of the Division of Nutrition, Food and Drug Administration, and Miss Jean McNaughton, Regional Nutrition Officer, Food and Agriculture Organization.

Dr. Kline, after giving a brief outline of the organization of the FDA, discussed the specific responsibilities of its Division of Nutrition. Prevention of adulteration and mislabelling were cited as being of prime importance. Vitamins, and food to which vitamins have been added, command major attention. The Division makes large numbers of routine analyses of composition of these products and conducts research on development of new analytical procedures. Prevention of fraudulent, misleading and unsubstantiated labelling claims also calls for a great deal of routine work. Research is also conducted to clarify subjects of current, scientific controversy, as for example the health effects of saturated versus nonsaturated fats.

Miss McNaughton described FAO activities which relate to Food Technology. Their program seeks to advance progress in the home and commercial food industries of FAO member countries, includes demonstration and extension activities, applied research projects, organization of international symposia, publication of international reviews and summaries and student training. (Their prominent international publications include World Fisheries Abstract, Food Additives Legislation, Food Additives Control and Symposium on Preservation by Ionizing Radiations).

After the speakers had finished, the membership discussed the National Meeting Program until long after the usual closing time.

—A. P. Sidwell

DIXIE

The Dixie Section enjoyed tours of the new Gordon Foods plant (a division of Sunshine Biscuits, Inc.) and the new State Farmers' Market, both located on the South Expressway near Atlanta, on May 15th.

Gordon Foods Tour

Gordon's is the world's largest and most modern plant for the production of potato chips and allied food products. Here the members saw the latest

equipment for deep fat frying of potato chips and related products, as well as storage rooms for the raw and finished products packed the "Magic-Pak" way.

Gordon Foods began business in March 1938 in Atlanta. The principal product was potato chips, with peanut butter sandwiches, salted nuts, and nut meats rounding out the initial line. All were made and packaged by hand. By 1941 the "Little Red Truck" —symbol of Gordon Foods' fresh-to-your-store distribution system—became familiar to thousands of people in Alabama, Florida, Tennessee, Mississippi, Arkansas, the Carolinas, and Indiana. There were some forty items in the Gordon line. End-of-the-war years marked the beginning of another era in the expansion program. Common stock in Gordon Foods, Inc., was offered to the public in 1947. Increased consumption of Gordon products was not the only concern of the company. They also recognized their obligation to insure their products reaching consumers with flavor and freshness intact. Gordon Foods' technologists, working with Research Chemists at Georgia Institute of Technology, developed Gordon's "Magic-Pak," which contains a product designed to absorb moisture within the bag of potato chips and assist in protecting their crispness.

The roof of the Gordon plant is covered with a 4-inch deep, 4½ acre "lake" which aids in cooling the plant area. The four latest type potato chip machines are completely automatic and can turn out from 3,700 to 4,000 pounds of finished chips per hour. Electric-Eye sorting machines automatically grade peanuts after blanching. All that are not properly blanched or any undesirable nuts are automatically rejected.

State Farmer's Market Tour

This, the largest farmer's market in the world, was opened for business in 1958. It has facilities for 80 wholesale produce dealers as well as for 1024 retail trucks which park under sheds. This market was constructed as a bond issue project authorized by the Georgia State Legislature. The total sales each year are from $38 to $42 million. Sixty per cent of the sales are outside a 100-mile radium of Atlanta, forty per cent in Atlanta. The market is a redistribution point for other areas, including New York and Canada. The market works closely with the Poultry Industry and other industries in the state. Ten per cent of the produce comes in by rail and ninety per cent by truck. Limitation on produce is 10% culls, only. Any produce having more than this amount of culls is thrown out.

A canning plant is operated by the State Department of Agriculture for the use of the housewives who can buy cheaper produce at the peak seasons and can it for home use. Fees are charged to cover cost of cans and operation only.

Mr. Boyce Dyer, director of the State Markets led the tour in the afternoon and told of the operation of the Farmers' Market after the evening meal. Mr. R. A. Bottomley, councilor of the Australia Northern Section brought "Greetings" from Australia to our section.

Election of officers for the Dixie Section was held. Officers elected for 1959–60 are: Chairman, William B. Cown, Ga. Tech., Atlanta; Vice-chairman, Ralph Bohn, consulting chemist, Atlanta; Sec.-Treas., Anjus J. Shingler, the Coca-Cola Co., Atlanta; Councilor, Dan E. Pratt, U. of Ga., Athens; Editor, H. Ray Malcom, Pomona Products Co., Griffin, Ga.

—Cordelia Ellis

N. CALIFORNIA

Dr. Harold W. Schultz, who is head of the Food and Dairy Technology Department, Oregon State College, Corvallis, Oregon, spoke to us on "Foods for Tomorrow's Market Basket" on June 3 at a joint meeting with the Food and Nutrition Section of the American Public Health Association at DiMaggio's Restaurant, San Francisco.

There are over 6000 different food items in the retail food stores today, Dr. Schultz remarked. In 1941 there were no more than 1500. This could mean that there are many more different food crops produced on our farms today than 15 to 20 years ago. But this is not true, for we still have one kind of milk, essentially, one kind of eggs, the same kinds of cereal grains and the same few kinds of meat animals we have had for many years. We do have a few more fruits and vegetables but these cannot account for the increased number of consumer items.

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The growth of the food industry and the vast improvements in our food supply have most certainly been due to the application of science and technology.

—S. V. Noland

MINNESOTA

The Minnesota Section of The Institute of Food Technologists was given a plant tour of the Rap-In-Wax Paper Company prior to attending a seminar on Flexible Packaging.

The plant tour made it possible for members to observe various printing processes in addition to the methods used in manufacturing breadwrappers, various foil structures, barrier materials and the well-known Rap-In-Wax Household Roll.

Packaging—A Fifteen Billion Dollar Business

P. M. Grieve, executive vice-president, Rap-In-Wax Paper Company, described the packaging business as a 15 billion dollar industry. Mr. Grieve stated that one of the fastest growing segments of the packaging industry was that of flexible packaging.

"In the past few years," continued Mr. Grieve, "the homemaker has been treated to one of the largest mass advertising attacks since the war, and this one was aimed at the idea of convenience in the home."

"Convenience in packaging, handling, storage, merchandising and preparation are important to flexible packaging manufacturers, food product manufacturers, retailers and to the ultimate consumer," said Mr. Grieve.

"Quality Control in Flexible Packaging"

Rogers T. Hefty, chief quality control engineer of the company, told of methods used to make it possible to produce quality flexible packaging materials continuously.

The Good Package

Donald D. Davies, creative art director, said that the biggest functional package he had ever worked on was the camouflaging of the "U. S. S. Suwanee" during World War II, with a 4 inch paint brush.

Mr. Davies listed several points which determine a good package: A good package stops the eye ahead of all competitive products on the shelf . . . conveys the feeling of the product it contains . . . has the most possible "impulse appeal" . . . inspires confidence in product and manufacturer . . . provokes instant recognition of both brand and product name . . . is easier to handle, store, open and use than competitive products . . . has a design personality strong enough to make people remember it and reach for it the next time they are in the store.

According to Mr. Davies, color, logotype, legibility, lay-out, key-line art are all partners in the development of a new or better package and require the element of enthusiasm on the part of the client to set the wheels in motion for an imaginative and creative artist. The client can best furnish enthusiasm to the artist by passing on the necessary background material and information to provide the fuel needed for developing creativeness.

"Sales Potential of Flexible Packaging"

A. H. Loux, commercial sales manager, said that in the last three years the packaging industry has had an over-all growth of about 5%, but that the flexible packaging segment has had an increase of about 33%. The sales potential of a new packaging material must also be evaluated in terms of cost, convenience, and merchandising. According to Mr. Loux, flexible packaging can lower packaging and shipping costs by being easier to handle and lighter in weight than rigid containers.

Frozen foods which can be heated in the oven, or by merely dropping a pouch into boiling water are two of the homemaker conveniences made possible by flexible packaging.

Flexible packaging—foil with its glitter, the pouch with its transparency, the wax papers with their gloss, the printing and the rotogravure full-color illustrations all combine to make the flexible packaging industry a leader in selling the products the food industry develops and produces.

Food Additives

Norman Foster, chief chemist, Minneapolis Office of the new Food and Drug Administration, gave a timely review of the new food additive law, particularly as it applied to food packaging materials.

During a brief business meeting the following members were elected to serve as officers: Chairman—Gerald Vacha, State Department of Agriculture, State of Minnesota; Vice-chairman—Howard Bauman, Biological Research Branch, The Pillsbury Company; Counselor (2 years)—John Wintermantel, General Mills; Secretary-Treasurer — Shirley Trantanella, Frozen Food Laboratory, Department of Horticulture, University of Minnesota.

—F. L. Sorensen, Jr.

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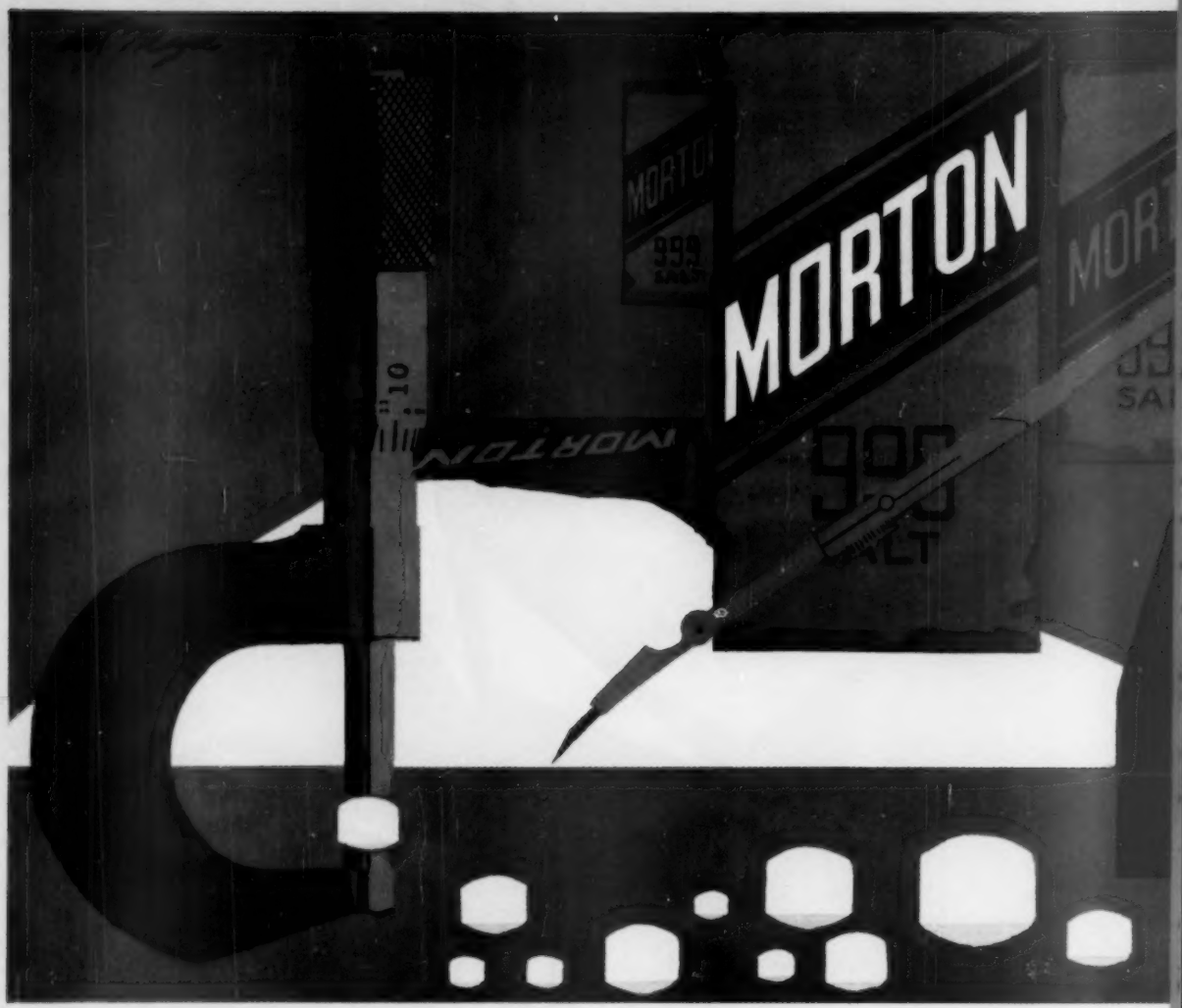

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Literature

BOOKS

ADVANCES IN FOOD TECHNOLOGY, Vol. 8. 1958. Ed. E. M. Mrak and G. F. Stewart. Academic Press Inc. Publishers, 111 Fifth Ave., New York, N. Y.

The eighth volume in this most valuable series is well up to the standard of its predecessors and serves to emphasize the enormous field which is now classed as Food Technology. Each of the contributors, three from the U.S.A., two from the United Kingdom and one from the Netherlands, is a recognised expert in his own field and, therefore, there is a general high quality of presentation throughout the book.

However, in spite of the general excellence there is one contribution which, to this reviewer, is a gem among gems. I refer to Dr. Amerine's splendid review of the inorganic constituents of wines. One cannot but admire the author's industry and scholarship in organizing the material from so many sources (519 references in six languages from 18 different countries) so concisely and neatly. This could well serve as a model to literature reviewers and its contents will be of considerable value to enologists and food analysts in general. It will, no doubt, be comforting to many readers to learn that one-third to one-half of one's daily requirement of several essential minerals can be obtained from a litre of wine.

Dr. Scott Blair in his review of "Rheology in Food Research" describes both the nature and extent of the problem and emphasises the need for competent specialists in this highly complex field. At the same time he reveals his own stature and outstanding contribution. He reviews the many empirical methods which have been developed to examine a variety of physical properties in foods and while all cereal chemists might not agree that the Swanson mixograph provides a " . . . highly successful rheological test used to predict the quality of a foodstuff" his comments and guidance are most helpful. No one, of course, is more able to discuss the fundamental studies of the rheological properties of foods and this section of the review is probably the most stimulating. While the author might have said more to discourage the dabbler and the knick-knack merchant, there is no doubt that his review will whet the appetite of the serious student for Dr. Scott Blair's earlier and more comprehensive works on this extremely important subject.

Dr. F. E. Lee presents a very useful survey of the literature under the title "The Blanching Process." He covers the compositional changes which occur in vegetables during steam and hot water blanching and discusses their effect on nutrients and on chlorophyll content and colour retention. He presents an interesting account of the progress made in electronic

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blanching. An odd sentence here and there (e.g. "Steam Pressure" p. 86) appears to have been added as an after thought and perhaps for the sake of completeness, some reference to the work of E.G.B. Gooding at Aberdeen, Scotland and that of T. S. Pang at Taiwan might have been made. Nevertheless, Dr. Lee's contribution will prove a most valuable work of reference for all who are concerned with the canning, freezing or dehydration of vegetables and fruits.

Dr. Max Milner's work is well known to all cereal chemists and his review of "New methods to detect and eliminate insect-infested grain" which includes a critical account of the major physical and chemical methods of detecting internal damage caused by insects in grain and proposals for separating the sound from the unsound kernels will be well received. The author is to be congratulated on the stand he takes in relation to the quantitative expression of insect contamination. His assumption that the insect particles are of the same overall dimensions as the particles of flour they contaminate may not be wholly valid in that only one dimension (e.g. the diameter of a leg) need be equal to or less than that of the screen aperture in order to pass through. This notwithstanding, his argument is sound and reasonable and deserves support and further investigation in those countries which base their limits of tolerance on a count per unit weight.

Dr. P. A. Roelofsen gives a thorough and learned account of the biochemical changes which take place during the fermentation, drying and storage of cacao beans. He discusses the micro-organisms responsible for the fermentation reactions and describes the changes which the polyphenols and other constituents undergo during each of the conditions to which the beans are subjected. His remarks on flavour and aroma serve to re-emphasize how little is known about this all important aspect of food technology. Dr. Roelofsen's own considerable and original contribution to the field he reviews is well illustrated.

For those who start at the beginning of Volume 8 and read on to the end the last contribution is well worth waiting for and constitutes a fitting climax. In just under 100 pages Dr. Hulme presents a scholarly account of the composition of and the changes which occur in apple and pear fruits on the tree and after harvesting. As the author suggests in his introduction, the contents will probably be of at least as much interest to the plant physiologist as to the biochemist.

It would be thoughtless to conclude without a word of congratulation to the editors, Drs. Mrak and Stewart, for maintaining the excellent level of organization and presentation which has persisted throughout the series and to the Editorial Board for the catholicity of the subject material and for the excellence of the contributors selected.

J. H. Hulse
Toronto, Ontario, Canada

LITERATURE ON FOOD RESEARCH AND FOOD TECHNOLOGY

Commodities

Checklist of U. S. standards for farm products. Revised. Washington, 1959. 16 p. (U.S.D.A. Marketing information division. AMS 210.) pap. apply.

Coffee for armed forces, military development and conversion to industry supply. Franz A. Koehler. Washington, 1959. 134 p. (U. S. Quartermaster General of the Army. Historical Branch. QMC historical study series II.

Another volume in the history of military development and procurement of food.

Grading and inspection of eggs and egg products. Revised. T. H. Pond and Lester Kilpatrick. Washington, U. S. Govt. Print. Off., 1959. 24 p. (U.S.D.A. Agricultural information bulletin 159.) pap. $0.15.

Economic effect of U. S. grades for beef. E. R. Bowman and F. C. Genovese. Washington, 1959. 199 p. (U.S.D.A. Marketing service reports 298.) apply.

Encyclopedia of candy and ice-cream making. Simon I. Leon. New York, Chemical Publishing Co., 1959. 454 p.

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[a] Material for this list should be sent to Dr. James G. Hodgson, Chief, Library Branch, Quartermaster Food and Container Institute for the Armed Forces, 1819 W. Pershing Road, Chicago 9, Illinois.

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An alphabetical listing of confections, ingredients and processes.

Meat we eat, 5th rev. ed. P. T. Ziegler. Danville, Ill., Interstate, 1950. 548 p. $5.00.

Shrinkage and grades of wheat stores in county elevators in Kansas. J. W. Taylor and R. E. Clifton. Washington, 1959. 14 p. (U.S.D.A. Agricultural marketing series AMS-291.) pap. apply.

Trends in the United States sugar industry, production, processing, marketing. Washington, U. S. Govt. Print. Off., 1959. 24 p. (U.S.D.A. Marketing research reports 294.) pap. $0.15.

Food Technology

Cleaning plastic equipment used in handling maple sap. C. O. Willits, H. A. Frank, and R. A. Bell. Washington, 1959. 12 p. (U.S.D.A. Agricultural research service, ARS 73-23.) pap. apply.

Correlation in radiation sterilized foods of chemical changes associated with characteristic flavor developed, April 22, 1955–June 30, 1956. Chicago, Quartermaster Food and Container Institute, 1956. 13 p. processed. (Contract DA 19-129-QM 407-5502, Report 9.) Now available as PB 131948 from the Office of Technical Services, U. S. Dept. of Commerce. $0.50.

Frozen foods. Kirby M. Hayes and David B. Barrow. Amherst, Mass., 1959. 15 p., processed. (University of Massachusetts. Extension Service. Leaflet R-5.) Tips to the store manager on how to get the most from frozen foods.

Getting the most out of frozen foods. Kirby M. Haynes and Charles E. Eshbach. Amherst, Mass., 1959. 24 p. (University of Massachusetts. Extension Service. Leaflet C-12.) A guide to the consumer in selection, storage and care of frozen foods.

Chemical Aspects

Analytical chemistry; some new techniques. A. G. Jones. New York, Academic Press, 1959. 276 p. $7.50.

Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto, 1957. Katashi Ichihara and others, eds. New York, Academic Press, 1958. 555 p. $16.50.

Safety of Polyoxyethylene (8) Stearate for use in foods. A report of the Food Protection Committee of the Food and Nutrition Board. Washington, 1958. 19 p. (National Research Council. Publication 646.) "There is no consistent indication of toxic activity by the material in experimental animals at a dietary level below 5 per cent. It is concluded that the use of polyoxyethylene stearate at levels not greater than 0.05 per cent in the human diet would be safe." Conclusions, p. 17.

Some tropical South Pacific island foods; composition and nutritive value. M. Murai and others. Honolulu, 14, University of Hawaii Press, 1958. 159 p. $2.00.

Type reactors in fermentation chemistry. Lowell L. Wallen, Frank H. Stodola and Richard W. Jackson. Washington, U. S. Govt. Print. Off., 1959, 496 p., processed. (U.S.D.A. Agricultural Research Service. ARS-71-13). paper. Largely the diagrammatic formulas of chemical compounds and their resulting changes due to the use of specific microorganisms, with bibliographic references to sources of information. Arranged by type of reaction.

Economic and Social Aspects

Food for the people of Australia. R. C. Hutchinson. Sydney, N.S.W., Angus and Robertson, 1958. 262 p. 32 s 6 d.

Grade names used in U. S. standards for farm products. U. S. Agricultural Marketing Service. Washington, 1958. 30 p. (U.S.D.A. Agricultural handbook 157.) Apply.

Promotion of lamb—results of a campaign in Cleveland, Ohio. T. S. Hunter, W. E. Clement and N. Havas. Washington, 1958. 58 p. processed. (U.S.D.A. Marketing research reports 292.) Apply.

Miscellaneous

Alcohol in Italian culture, food and wine in relation to sobriety among the Italians and Italian Americans. Giorgio Lolli and others. Glencoe, Ill., Free Pres, 1958. 155 p. $4.00. One of the Yale Center of Alcohol Studies. Scientific reports on the relationship between eating and drinking among Italians and Italian Americans and the absence of drunkenness.

Man's place in nature. Thomas H. Huxley. Ann Arbor, Mich., University of Michigan Press, 1959. 184 p. (Ann Arbor paper backs, AA 24.) pap. $1.75. One of a new series of paperback editions which needs to be watched because it includes many of the basic works of science.

National strategy in an age of revolutions: Addresses and panel discussions of the Fourth National Military-Industry Conference, Feb. 17-19, 1958, Chicago. Ed. by George B. de Husgar. New York, Frederick A. Praeger, 1959. 282 p. $6.00. Food plays a part in the strategy.

Origin of cultivated plants. Alphonse de Condelle. New York, Hafner Pub. Co., 1959. 476 p. A reprint of the second edition of 1886. Includes many of the plants which serve as sources of foods.

Science Information News, v. 1, no. 1, Feb.-Mar. 1959. Washington, National Science Foundation, 1959 +. A new periodical with news of the scientific field issued by the National Science Foundation.

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ABSTRACTS

General Foods Research Center
Selected by H. A. Campbell

ANALYTICAL METHODS

Food. Review of applied analysis.

MORGAREIDGE, K. (Food and Drug Research Labs., Inc., Maspeth, N. Y.). *Anal. Chem. 31*, 691–6 (1959).

This review covers advances in food analysis represented by publications available to the author prior to December 1, 1958. Excepting the more spectacular achievements resulting from the application of the more advanced chromatographic methods, particularly in the vapor phase, progress in food analysis has been largely a matter of refinement of previous methods or their application to new situations.

Starch electrophoresis. I. Starch block electrophoresis.

BLOEMENDAL, H. (Netherlands Cancer Inst., Amsterdam, The Netherlands). *J. Chromatography 2*, 121–35 (1959).

Starch block electrophoresis is of growing importance as a useful tool in protein chemistry for analytical studies as well as for isolation on preparative scale. The starch block technique opens up the possibility for sepn. of various protein mixts.; it is often successfully applied in combination with other isolation methods and, in some cases, gives good results where other fractionation procedures fail. 62 references.

Detection and measurement of cis unsaturation in fatty acids.

HOLMAN, R. T., ENER, S. AND EDMONDSON, P. R. (Dept. of Physiol. Chem., Univ. of Minnesota, Austin). *Arch. Biochem. Biophys. 80*, 72–9 (1959).

The absorbance of fatty acids at 2.15 μ has been related to the unsatn. of the samples and found to be a measure of the *cis* unsatn. Moles *cis*/kg. = 0.76 ($k \times 10^4$) — 0.96. Absorbance of several fatty acids contg. other types of unsatn. indicates they absorb negligibly at this wavelength. A comparison between the direct measure of *cis* unsatn. and its measurement by iodine value minus *trans* unsatn. (infrared) has been made on several com. hydrogenated food prepns. and on soybean oil at several stages of hydrogenation.

BIOLOGICAL SCIENCES

Biochemistry

Chromatography of dipolar ions. Activity coefficients of amino acids and peptides in ion exchange resins.

FEITELSON, J. (Dept. of Phys Chem., Hebrew Univ., Jerusalem, Israel). *Arch. Biochem. Biophys. 79*, 177–86 (1959).

The distribution of amino acids and peptides between a sulfonated polystyrene ion exchange resin and the ambient soln. was detd. from chromatography. This together with the exchange and ionization equilibria involved permitted the comparison of activity coeffs. of the tonin precipitable sterol vitamins and in soln. Some factors governing the resin-solute interaction are discussed.

Ultraviolet spectrophotometric method for the determination of cholesterol.

WEIGENSBERG, B. I. AND MCMILLAN, G. C. (McGill Univ., Montreal, Can.). *Am. J. Clin. Pathol. 31*, 16–25 (1959).

A spectrophotometric method is described for the detn. of cholesterol, either in its free form or as its digitonide. The technic is based upon the absorption of ultraviolet light in the 204 to 206 mμ region by the ethylene linkage in the B ring of cholesterol. The method is suitable for determining the free and total cholesterol in serum and tissues except in the presence of high concn. of digitonin precipitable sterol vitamins and hormones. The cholesterol does not react with any reagents except digitonin and can be reclaimed.

A method for simultaneous catheterization of major hepatic vessels in a chronic canine preparation.

SHOEMAKER, W. C., WALKER, W. F., *et al.* (Harvard School of Public Health, Boston, Mass.). *Am. J. Physiol. 196*, 311–14 (1959).

A feasible method for measuring concn. changes of various substances as they enter and leave the liver is described together with the operative technique. This prepn. allows simultaneous sampling of blood from all major hepatic vessels in the *in vivo*, unanesthetized dog under chronic exptl. conditions.

Structure of α-keratin.

FRASER, R. D., MACRAE, T. P. AND ROGERS, G. E. (CSIRO, Melbourne, Australia). *Nature 183*, 592–4 (1959).

The molecular structure of α-keratin was investigated by combining electron microscope observations with parallel studies of low-angle X-ray diffraction and measurements of infra-red dichroism. Earlier conclusions that α-keratin consists of microfibrils about 60–80 A. in diam. embedded in a matrix rich in sulfur have been confirmed. It is concluded from the present expts. that during hydration the microfibril lattice expands to a greater extent than does the interchain distance within the microfibrils.

The reductive cleavage of disulfide bonds and its application to problems of protein structure.

SELA, M., WHITE, F. H. AND ANFINSEN, C. B. (Natl. Insts. of Health, Bethesda, Md.). *Biochim. et Biophys. Acta 31*, 417–26 (1959).

The quant. reduction of disulfide bonds in ribonuclease and lysozyme may be achieved at room temp. with thioglycolic acid in 8 *M* urea at pH 8.5. Sulfhydryl groups of the resulting cysteine residues may be reacted with iodoacetic acid to yield the S-carboxymethyl deriv. of these residues. The S-carboxymethylcysteine content of reduced and alkylated proteins may be estd. by dinitrophenylation of acid hydrolysates followed by chromatographic isolation of DNP-SCMC. The method described is of particular value as an initial step in the study of amino acid sequence in such tryptophan-rich proteins as lysozyme.

On the biological methylation of histamine. 3.

LINDAHL, K. M. (Inst. of Zoophysiol., Univ. of Uppsala, Sweden). *Acta Chem. Scand. 12*, 2050–1 (1958).

Histamine-2-^{14}C-dihydrochloride was incubated for 1 hour at 37° C. with an enzyme ext. obtained from mouse liver, and the resulting mixt. analysed. The authors conclude that the sol. fraction of mouse liver contains a histamine methylating enzyme which utilizes *S*-adenosylmethionine as methyl donor with great efficiency.

Catabolism of physiological quantities of histamine in vivo.

SCHAYER, R. W. (Merck Inst. for Therapeutic Research, Rahway, N. J.). *Physiol. Revs. 39*, 116–26 (1959).

The *in vivo* mechanisms of histamine catabolism are reviewed in the light of work done since 1954. A detailed discussion of oxidation, methylation, acetylation pathways and of the quant. detn. of a number of metabolites is included. The author emphasizes the need for a satisfactory system of nomenclature in this area.

Microbiology

A rapid membrane filter method for direct counts of microorganisms from small samples.

ECKER, R. E. AND LOCKHART, W. R. (Dept. of Bacteriol., Iowa State Coll., Ames). *J. Bacteriol. 77*, 173–6 (1959).

Several methods have been described by which membrane filters may be used in making total counts of the microorganisms in fluid samples. When a sample is filtered the cells are well distributed on the filter surface of known area. The cells are stained and counted by direct microscopic observation, after the filter has been made transparent by application of immersion oil. The technique described here, using simple app. and few

manipulations, is useful in situations which require the examn. of many small samples at frequent intervals.

Studies of the toxin of Clostridium botulinum. V. Prophylactic immunization of pheasants and ducks against avian botulism.

BOROFF, D. A. AND REILLY, J. R. (New England Inst. for Medical Research, Ridgefield, Conn.). *J. Bacteriol. 77*, 142–6 (1959).

The results of the expts. indicated the feasibility of prophylactic immunization of pheasants and ducks with the establishment of an active immunity against as much as 2000 LD_{50} bird doses of toxin. The impression was that toxoid-incomplete adjutant mixt. was a better antigen than toxoid alone, and that 3 to 4-week intervals between the first and second immunizing injections resulted in a greater degree of resistance. The active immunity so induced lasted at least 8 months. The fact that immunization may be started when the birds are 1 week old is of considerable advantage.

Effect of chlorpromazine on Salmonella enteritidis infection in mice.

GROSZ, H. J. AND NORTON, J. (Purdue Univ., Lafayette, Ind.). *Science 129*, 784–5 (1959).

Chlorpromazine increases the susceptibility of mice to infection with *Salmonella enteritidis.*

Nutrition

The genesis and absorption of intestinal ammonia.

ANON. *Nutrition Revs. 17*, 38–40 (1959).

Studies indicate that ingested proteins may vary greatly in their ability to elevate blood ammonia concns. Also, the liberation of ammonia, possibly from amide nitrogen, independent of bacterial action may be responsible for the prompt rise in portal vein ammonia after protein feedings.

Biotin in protein synthesis.

ANON. *Nutrition Revs. 17*, 21–2 (1959).

The results obtained are consistent with the idea that biotin does not participate directly in protein synthesis but acts, perhaps as a coenzyme, in the synthesis of dicarboxylic acids of the citric acid cycle which are essential for the processes of oxidative phosphorylation, which, in turn, secure the energy requirements for protein synthesis.

Angiotensin.

PAGE, I. H., BUMPUS, F. M. AND SCHWARZ, H. J. *Sci. American 200*, 54–8 (Mar. 1959).

Recently, a substance called angiotensin was isolated from blood. This is a peptide material consisting of a chain of 10 amino acids which can raise the blood pressure. It is suggested that hypertension might be controlled by synthesizing a peptide similar to angiotensin but lacking its physiological activity.

Action of vitamin A.

ANON. *Nutrition Revs. 17*, 22–4 (1959).

It appears that the protein opsin, and consequently the outer segments of the rods of which it is an important constituent, are stabilized by combination with retinene and deteriorate rapidly in its absence. The authors suggest that many tissues contain structural proteins which are also stabilized by combination with vitamin A or its derivs. These also deteriorate in the deficiency state. If this is true, a logical general explanation is finally available for the function of a previously little understood vitamin.

Rate of oxidation of lipids.

ANON. *Nutrition Revs. 17*, 17–9 (1959).

The rapid disappearance rate of the triglycerides from the blood may represent the speed with which they are utilized by the body. The slow disappearance of the phospholipid fraction suggests that it follows a metabolic pathway that is different from that of the triglycerides and that phospholipids remain in an intact state in the blood for fairly long periods of time. It is suggested that the phospholipid component of the chylomicrons forms a reservoir within the plasma and lymph which is used over and over again in forming an emulsifying surface around the triglyceride particles.

Dietary fat, obesity and longevity.

ANON. *Nutrition Revs. 16*, 350 (1958).

Recent expts. conducted in a study employing over 600 animals emphasize again that, at least in rats, increased growth rate leading to obesity can be brought about by a high fat diet with no other manipulation and that these conditions may lead to a decreased life span.

Essential fatty acids in infant nutrition. II. Effect of linoleic acid on caloric intake.

ADAM, D. J., HANSEN, A. E. AND WIESE, H. F. (Dept. of Pediatrics, Univ. of Texas School of Med., Galveston). *J. Nutrition 66*, 555–64 (1958).

The Caloric intake was measured on 18 healthy infants under 4 months of age who, for 1 to 28 weeks, were fed on 1 or several of 12 different types of milk. The linoleic acid intake ranged from $<0.1\%$ to 8.0% of the total Calories. In spite of differences of 20 to 40% in Caloric intake the slope of the wt. curve did not change in the majority of the infants studied. On the basis of the data presented it appears that in young infants optimum Caloric efficiency is attained when linoleic acid comprises about 4% of the Caloric intake.

Effect of mixed fat formula feeding on serum cholesterol level in man.

HASHIM, S. A., CLANCY, R. E., HEGSTED, D. M. AND STARE, F. J. (Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.). *Am. J. Clin. Nutrition 7*, 30–4 (1959).

Either the safflower oil or the mixt. of safflower and coconut oils caused a marked decrease in serum cholesterol in humans. The results are incompatible with the proposed hypotheses that the serum cholesterol-lowering effect of a dietary fat is proportional to the iodine value or the linoleic acid content of the fat. In addn., the results do not support the view that the satd. fats counteract the effect of polyunsatd. fats.

Effect of low-calcium intake on phosphorus retention.

JOHNSTON, F. A. AND THORANGKUL, D. (New York State Coll. of Home Econ., Cornell Univ., Ithaca). *J. Am. Dietetic Assoc. 35*, 31–3 (1959).

Seven young men, 3 accustomed to daily phosphorus intakes of 1800 to 2050 mg./day and 4 to intakes of 900 to 1600 were placed on a moderate intake of phosphorus for 95 days. Balance periods of 16 days were conducted at the beginning, middle and end of the study. The mean phosphorus intake was 1063 mg./day and calcium 232 mg. The percentage of phosphorus absorbed by the 7 subjects was constant at from 75 to 78. All except one subject was in neg. balance at the end of the study. The rate of loss was higher for those subjects accustomed to high intakes.

Adaptation to a low-calcium intake.

THORANGKUL, D., JOHNSTON, F. A., KIME, N. S. AND CLARK, S. J. (New York State Coll. of Home Econ., Cornell Univ., Ithaca). *J. Am. Dietetic Assoc. 35*, 23–30 (1959).

Five men accustomed to intakes of 350 to 750 mg. calcium/day and 6 accustomed to intakes of 1250 to 2000 mg. were placed on a balance study in which calcium ranged from 175 to 253 mg./day. The mean urinary loss of calcium by the low-calcium group was 91 mg./day, while the high-calcium group lost 175 mg. during the first 16 day period. Continuation of the study for 95 days indicated a small trend towards increased retention. There was no indication that 180 to 230

mg. of calcium/day was adequate. Bone density tests and urinary excretion studies suggest a difference in stores due to habitual calcium intake.

Hypoglycemic effects of saccharin in experimental animals.

THOMPSON, M. M. AND MAYER, J. (Harvard School of Public Health, Boston, Mass.). *Am. J. Clin. Nutrition 7*, 80–5 (1959).

A study was made of both chronic and acute administration of saccharin to obese-hyperglycemic mice, their lean littermates, and adult female Wistar rats. Hypoglycemia was produced in obese-hyperglycemic mice following chronic administration of 125–175 mg. saccharin daily; no change in blood sugar was seen in the other animals.

The diet of sledge dogs.

TAYLOR, R. J. F., WORDEN, A. N. AND WATERHOUSE, C. E. (Univ. of Cambridge, Engl.). *Brit. J. Nutrition 13*, 1–16 (1959).

The sledging diet for dogs was found to consist of a daily allowance of 1 lb. of dog pemmican animal and a dried coned. food made mainly from beef. It is concluded from chem. analyses and dietary studies, that pemmican is an ill-balanced diet, with an abnormal and wasteful ratio of protein to carbohydrate. A diet believed to be more suitable for use in the field has been devised and is in use.

Toxicology

Feeding test with carrageenin.

NILSON, H. W. AND WAGNER, J. A. (U. S. Bureau of Com. Fisheries, College Park, Md.). *Food Research 24*, 235–9 (1959).

The data indicate that carrageenin is free from any deleterious substances which adversely affect the health of rats and mice or utilization of nutrients, except possibly for mechanical interference when excessively high levels are fed.

Gibberellic acid: toxicologic and pharmacologic studies.

KIMURA, E. T., YOUNG, P. R. AND STANISZEWSKI, K. (Abbott Labs., Chicago, Ill.). *J. Am. Pharm. Assoc. 48*, 127–9 (1959).

The plant growth stimulant, gibberellic acid, was tested for its toxicologic and pharmacologic properties in mice, rats, cats, guinea pigs, rabbits, and dogs. Both subacute toxicity in mice, and subchronic toxicity in dogs and rats showed the compd. to be asymptomatic and free of pathologic changes, histologically. The following studies were negative: circulation and respiration, barbiturate potentiation, antipyresis, anticonvulsant activity, intestinal motility, blood cholesterol, and blood glucose.

Chemicals in foods.

DEEDS, F. (Western Utilization Research and Devel. Div., Albany, Calif.). *J. Am. Dietetic Assoc. 35*, 19–22 (1959).

The wide variety of minor chem. constituents occurring naturally in foods and the diverse physiologic effects produced by those that have been investigated emphasize: the need for a systematic investigation of this relatively unexplored field of research; the desirability of recognizing that these minor chem. constituents may be fully as important to the health of the consumers as are chem. additives; and that the dietitian should be as interested in these constituents as in chem. additives.

Possible toxic factor of trichloroethylene-extracted soybean oil meal.

MCKINNEY, L. L., PICKEN, J. C., *et al.* (Northern Utilization Research and Devel. Div., Peoria, Ill.). *J. Am. Chem. Soc. 81*, 909–15 (1959).

Treatment of amino acids and peptides with trichloroethylene, under conditions known to produce the bovine aplastic anemia-causing factor in soybean oil meal and other proteins, showed that trichloroethylene reacted readily with the sulfhydryl groups of cysteine and reduced glutathione. The S-dichlorovinyl derivs. of L-cysteine and L-glutathione were synthesized and found to produce the aplastic anemia syndrome in calves typical of that produced by trichloroethylene-extd. soybean oil meal. Enzymic hydrolysis of a toxic protein from trichloroethylene-extd. soybean oil meal resulted in the loss of a large portion of its toxicity. Chromatographic studies on the hydrolyzate indicated the presence of products resembling the synthetic compds.

FOOD AND FOOD TECHNOLOGY

Baking and Bakery Products

Taste and texture evaluation of lysine-supplemented baked products.

EHLE, S. R., HAUSE, N. L. AND PAUL, D. E. (E. I. duPont de Nemours and Co., Inc.). *Cereal Sci. Today* 74–6 (1959).

No off-flavor resulted from the addn. of nutritionally optimum amts. of L-lysine monohydrochloride to breads, cakes, and doughnuts. The only physical property altered by these addns. was crust color of breads. Although the crust was slightly darker for the lysine-fortified breads baked under the standard set of conditions used in these tests, the desired degree of browning was obtained by appropriate adjustments in baking temp. and time without affecting other properties.

Cocoa and Cocoa Products

The estimation of lecithin in chocolate coatings.

BREHM, J. B. *Mfg. Confectioner 39*, 17–18 (Mar. 1959).

An easy to use method for the estn. of the lecithin content of coating chocolate is presented. The method involves taking viscosity readings after known and decreasing amts. of lecithin are added to the chocolate. Viscosity data is plotted and the curves compared with those of known lecithin content. The author believes this method will also apply to any additive which reduces chocolate viscosity.

Coconut

The chemical composition and nutritive value of cocoanut and its products.

KRISHNAMURTHY, K., RAJAGOPALAN, M., *et al.* (Central Food Technol. Research Inst., Mysore). *Food Sci.* (India) *7*, 365–70 (1958).

A review is given of the literature on the chem. compn. of copra, and coconut oil, proteins, carbohydrates, cake and water. The manuf., use, and nutritive value of these products is also discussed.

Dehydration

Drying with superheated steam-air mixtures.

CHU, J. C., FINELT, S., HOERRNER, W. AND LIN, M. *Ind. Eng. Chem. 51*, 275–80 (Part I, Mar. 1959).

Granular sand was dried in an exptl. dryer using air, superheated steam and a mixt. which varied from 20 to 70% steam. Under the same conditions of mass flow and temp., superheated steam gave a faster rate than air during the constant rate period. Mixtures greater than 50% superheated steam showed no appreciable gain in drying rate during the constant rate period. During the falling rate period, steam gave a slower drying rate than air.

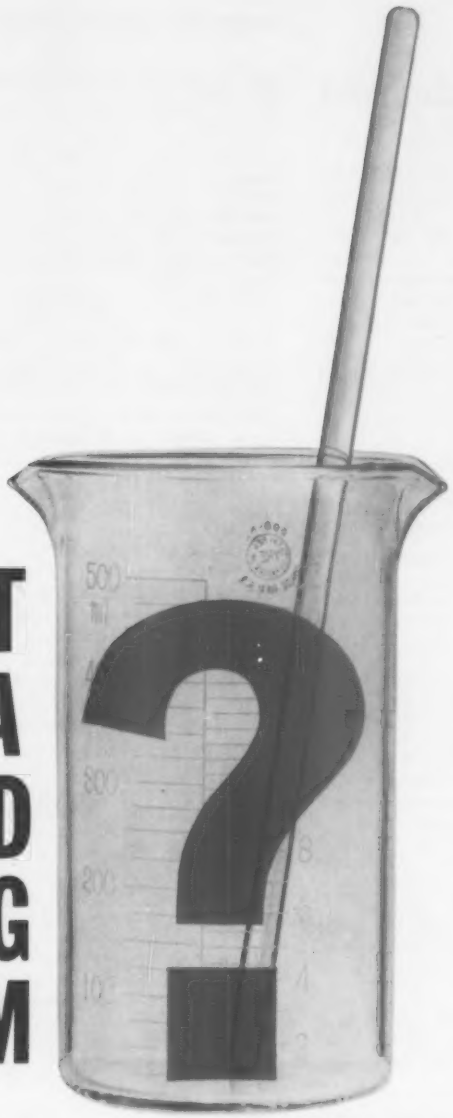
Refrigerate dried fruit to save quality.

MELEHAN, J. *Canner/Packer 128*, 32–3 (Feb. 1959).

Mayfair Packing Company finds storage at 32–35° F. prevents deterioration and loss of color of dried fruits. Low humidities are preferable. Retention of vitamin C was appreciably better in cold storage samples and acceptability of cold storage samples held for 2 years was equal to the incoming crop.

Fats and Oils

The origin of the marine polyunsaturated fatty acids.



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KELLY, P. B., REISER, R. AND HOOD, D. W. (Dept. of Oceanography, Texas A. & M., College Station). *J. Am. Oil Chemists' Soc. 36*, 104–6 (1959).

It was found that both shrimp and crabs lost much of their polyunsatd. acids on the fat-free diet and regained it again by ingestion, as do fish. The shrimp, however, appeared to synthesize more highly unsatd. acids from cottonseed oil than did other aquatic animals. The importance of the detn. of the structure of aquatic plant and animal fatty acids in the problem of the origin of the acids and their mechanism of synthesis was discussed.

Feedstuffs

Nutritive value of low and high protein sorghum grains for growing chickens.

VAVICH, M. G., KEMMERER, A. R., NIMBKAR, B. AND STITH, L. (Dept. of Agr. Biochem., Univ. of Arizona, Tucson). *Poultry Sci. 38*, 36–40 (1959).

The biol. value of the protein of 2 sorghum grains, contg. 10.5% and 15.3% protein, was compared using growth of chicks as the criterion. The protein of the low protein sorghum grain was found to be superior to the protein of the high protein sorghum. Microbiological amino acid assays showed that sorghum grains are low in lysine and arginine.

Milk

Radiation preservation of milk and milk products. V. Precursors to the radiation-induced oxidation flavor of milk fat.

HOFF, J. E., WERTHEIM, J. H. AND PROCTOR, B. E. Massachusetts Inst. of Technol., Cambridge). *J. Dairy Sci. 42*, 468–75 (1959).

Oxidation flavor produced upon irradiation of milk fat is derived from certain of its component fatty acids. One of these acids has been identified as linolenic acid. Other acids required for production of irradiation-induced oxidation flavor have been isolated in an unidentified mixt. This unknown isolate is believed to contain the vinyl group and branched chains. Linolenic acid produced a fishy flavor upon irradiation, while the mixt. of fatty acids contg. the vinyl group and branched chains gave rise to a flavor characterized as candle-like. These 2 flavors blended upon mixing to give the typical tallowy oxidation flavor of irradiated milk fat.

Poultry

Estrogenic residues in the edible tissues of stilbestrol-fattened chickens.

UMBERGER, E. J., GASS, G. H., *et al.* (U. S. Dept. of Health, Educ. and Welfare, Washington, D. C.). *Poultry Sci. 38*, 118–28 (1959).

The edible tissues of chicken treated with stilbestrol in the form of a 12-mg. pellet, a 15-mg. paste, and a 15-mg. liquid-rosin prepn. contain measurable amts. of estrogenic activity 4 to 5 weeks after treatment. Cooking does not destroy the estrogenic residues in the tissues.

Starch

Solution properties of dialdehyde starch.

LEVINE, S., GRIFFIN, H. L. AND SENTI, F. R. (Northern Utilization Research and Devel. Div., Peoria, Ill.). *J. Polymer Sci. 35*, 31–42 (1959).

Low levels of periodate oxidation increased the dispersibility of cornstarch in water at 98° C. Above the 10% level of oxidation, cornstarch became more resistant, and longer heating times were generally required to form stable solns. Introduction of dialdehyde groups into the anhydroglucose residues of the starch molecules thus made the structure labile toward degradation on heating in water. The observation that aggregation could be prevented by addn. of glucose was explained by competition of hydroxyl groups of glucose with hydroxyl groups of starch in their reaction with aldehyde groups.

The effect of salts upon starch granule rupture.

WINTER, S. S. AND LOISELLE, A. A. (Dept. of Chem., Northeastern Univ., Boston, Mass.). *J. Colloid Sci. 13*, 576–83 (1958).

Viscosity measurements show that salts do not affect the rate of starch granule rupture. This indicates that only the solvent plays a major role in the swelling process. The salts affect the conformations of the hydrated starch molecules in the same manner in which electrolytes affect other polyelectrolytes.

Substrate cleavage point of the α- and β-amylases.

MAYER, F. C. AND LARNER, J. (Noyes Lab. of Chem., Univ. of Illinois, Urbana). *J. Am. Chem. Soc. 81*, 188–93 (1959).

Hydrolysis of glycogen by cryst. β- and α-amylases has been carried out in H_2O^{18}. Enzymatically formed saccharides contained O^{18} in the 1 position to the extent of about 90%. Maltose controls contained only traces of O^{18}. It is concluded that both β- and α-amylases rupture the C-1 O bond of the α-1,4 linkages hydrolytically. A mechanism consistent with these findings is presented and discussed.

Industry

PERSONNEL

Dr. Samuel Cate Prescott (center) who, with W. Lyman Underwood, grandson of the founder of the William Underwood Company, first successfully placed food canning on a scientific basis in the 1890's, at dedication of the new Underwood-Prescott Laboratory at the Watertown, Mass. plant of the William Underwood Company. Participating in the ceremony is (left) George C. Seybolt, president of the Company, and L. V. Taylor (right), manager of product development. The dedicatory plaque just outside the research laboratory displays an industry tribute made to Messrs. Underwood and Prescott some years ago. The tribute reads: "The canners of this country owe more to Prescott and Underwood for placing their industry upon a scientific basis than to any other investigators."

George K. Parman has been promoted to the position of Technical Director of the Vitamin Division, Hoffman-La Roche, Inc. At the same time **Paul E. Sleezer** has been advanced to the position of Assistant Technical Director. Their new assignments began May 1. Both of these men have had long experience with the technical group of the Roche Bulk Vitamin Division.

G. K. Parman

Mr. Parman has spent his entire professional career in the pharmaceutical and food industries. A native of Nebraska, he graduated with a B.S. degree from Iowa State College. Mr. Sleezer is from Illinois and a graduate of the Illinois Institute of Technology, Chicago.

Dr. B. S. Schweigert is one of several Chicago area scientists who will lecture in the new course on nutrition offered at University College, University of Chicago, this fall. Latest trends in nutrition research will be discussed by experts; the course will be at the graduate level. Food industry technicians, biochemists, dieticians, nurses and many other groups concerned with nutrition will find the course a means of up-dating their knowledge.

B. S. Schweigert

The appointment of **Mr. John A. Kooreman** as Manager, Technical Sales Service, Food Division of Penick & Ford, has been announced by Dr. J. E. Killinger, Director of Technical Sales Service. Mr. Kooreman has been associated with Penick & Ford since 1939, specializing in the application of the company's products to the confectionery, ice cream, canning, baking, and allied fields. Mr. Kooreman is a graduate of Central College, Pella, Iowa, and Iowa State College, Ames, where he received a Bachelor's Degree in Chemical Engineering.

John A. Kooreman

National Biscuit Company has appointed **Miss Eleanor M. Crozier** director of Home Economics, it has been announced by Harry F. Schroeter, Director of Nabisco's General Advertising Department. Miss Crozier succeeds Mrs. Erna Nibley, who has resigned. Miss Crozier came to Nabisco in 1957 as Manager of Consumer Information. Previously she had served as Director of Home Economics for Minute Maid Corporation and her former associations include United Fruit Company, Good Housekeeping Magazine, and Hoffman-La Roche, Inc.

Aluminum Company of America, an exhibitor at the Institute of Food Technologists Philadelphia meeting, has announced the appointment of **Dr. Bernard Oser**, a leading toxicologist, as consultant on coatings for aluminum foil. He will advise on proper non-toxic materials to be used in coating processes involved in preparing foil for ultimate use in food packages. Dr. Oser, a graduate of the University of Pennsylvania, earned his Master of Science degree at Pennsylvania, and received his Doctor of Philosophy degree from Fordham University.

Dr. Lloyd A. Hall, Technical Director, The Griffith Laboratories, Inc., Chicago, Illinois, received the Honorary D. Sc. degree from Howard University, Washington, D. C. on June 5, 1959.

An unusual contribution to gas chromatographic analysis is an analog computer announced by Podbielniak, Inc., 341 E. Ohio St., Chicago 11.

To aid the analyst and speed up his efficiency, as well as to insure accuracy, the newly announced PODBIELNIAK COMPUTAGRAM now makes it possible to calculate a complete analysis in less than THREE MINUTES. Calculated percentages are accurate to within 0.1%. The computer corrects peak areas or peak heights for differences in the response of the detector and normalizes the corrected areas or heights to total 100, thereby giving the per cent (mole or weight according to correction factors inserted) of each individual component.

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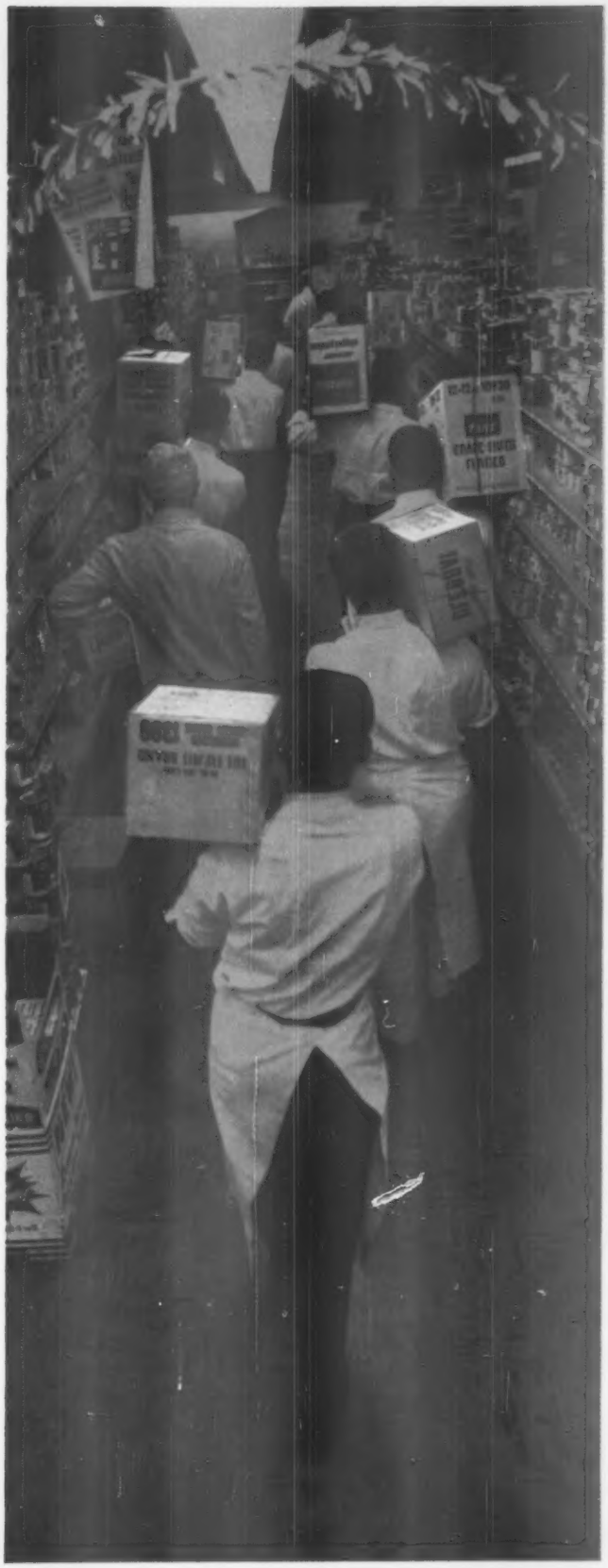

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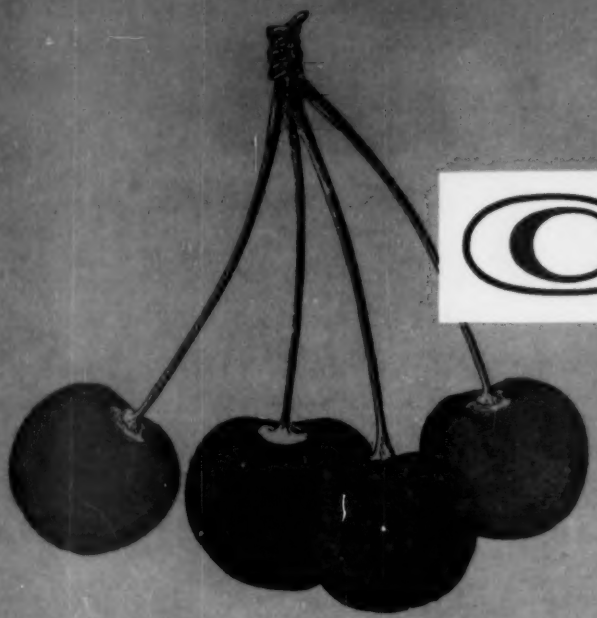

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